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Field and Laboratory Tests with B.T.B. Blowfly Dressing and Its Modifications, with Special Reference to B.K.B.

*By D. F. Waterhouse, M.Sc., A.A.C.I.**

Summary.

Laboratory and field trials demonstrated the superiority over other dressings of B.T.B. 15, the boric acid—tar oil—bentonite dressing of Lennox (1941). Since the recommended tar oil had become unavailable owing to war-time demands, it was replaced by a mixture of equal parts of power kerosene with either creosote 259 or middle oil 43. These were satisfactory substitutes except that the contact toxicity of the dressings was low. A dressing, B.K.B., containing 20 per cent. of a mixture of ortho-dichlorbenzene, lysol, and kerosene is described; it has a very high contact toxicity, stability, and powers of penetration, and is not irritant to the sheep.

Tests with a number of bentonites demonstrated the superiority, as a constituent of B.T.B., of processed American clays over all other samples.

1. Comparison of B.T.B. with Other Dressings.

Preliminary results with two boric acid—tar oil—bentonite mixtures (B.T.B. 30 and 15, containing 30 per cent. and 15 per cent. boric acid respectively) were reported by Lennox (1941). In the present series of tests, six of the most promising dressings available were selected for comparison with them. They were:—

C.B.E. (Freney and Graham, 1939). Recommended by the Council for Scientific and Industrial Research;

Sodium pentaborate† (Freney, unpublished);

Zinc sulphate dressing (Keast, 1939). Recommended by N.S.W. Department of Agriculture;

and three widely used commercial dressings.

(i) *Methods of Testing.*

(*a*) Penetration was tested in moist fleece directly over a strike, and also in long and short dry wool. Differences between dressings in ease of penetration were generally accentuated by using dry fleece. Methods involving time for complete immersion of weighed staples of unscoured wool in a dressing were not satisfactory owing to the large and unavoidable variation between staples.

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*An officer of the Division of Economic Entomology.

†Prepared by mixing 80 g. NaOH, 618 g. H_3BO_3, 3090 ml. H_2O.

(*b*) Contact toxicity tests were carried out as described by Lennox (1940), prepupae of *Lucilia cuprina* being immersed in the dressings for periods of 5 and 10 minutes, removed and allowed to drain and then placed in dry sand.

(*c*) Insectary tests were carried out in the following manner:—Struck sheep from the field, or sheep with artificial breech strikes produced by exposing them with wetted breeches to a high *L. cuprina* population, were used. The strikes were generally dressed without shearing the wool from the struck area. In some experiments, however, the dressing was applied after removing the wool, which gave a less severe test for the dressing. The sheep were then exposed to a high population of gravid *L. cuprina* in an insectary (J.B.C. 1933) where temperature and humidity favourable for oviposition were maintained. Site of oviposition and number of egg batches laid were recorded, and the areas were examined at intervals to determine whether or not the young larvae established themselves. The experiments were continued until restrike occurred on all dressings, although not necessarily on every individual sheep.

(ii) *Results.*

Results of laboratory and insectary tests are summarized in Table 1. Field tests were performed with all dressings, and, in addition, B.T.B. 15 was used extensively for 2½ years on an experimental flock of about 300 sheep in which there was a very high incidence of strike (40 to 70 per cent.).

(*a*) *Penetration.*—Five of the dressings penetrated struck wool satisfactorily. On application they were drawn into the fleece and needed little or no manipulation to disperse them over the struck area. Commercial dressing No. 3 penetrated fairly well but tended to sit on the surface, requiring some manipulation to disperse it evenly. Sodium pentaborate was absorbed fairly well by moist fleece, but owing to its very liquid nature, ran off readily. It was difficult to impregnate the strike area with this dressing without a fair amount of manipulation. The zinc sulphate dressing, a fairly thick paste, sat on the surface of the wool, had very poor powers of penetration, and required far more working in than the other dressings.

(*b*) *Effect on maggots in the strike.*—The most spectacular effect on the maggots was obtained with commercial dressings 1 and 2, for no living larvae were present six hours after application. Dressing No. 1 was apparently very irritant to the larvae, since they left the strike in large numbers as soon as the dressing was applied. C.B.E. and both B.T.B's. had about the same effect; strikes examined six hours after dressing occasionally had a few live maggots wandering about. Sodium pentaborate affected larvae slowly; many were alive 24 hours after dressing, but after about 40 hours no living larvae could be seen and a very large number of dead maggots could be found in the fleece over the dressed area. The zinc sulphate dressing produced a number of dead or moribund larvae six hours after application. In eighteen hours, some larvae did not seem to be much affected and continued to inhabit the strike wound until fully developed. Presumably

the initial contact effect of the dressing passed off with the evaporation of the carbon tetrachloride in the first few hours after application, and the zinc sulphate had insufficient stomach toxicity to inhibit development. Strikes dressed with this preparation frequently needed more than one application to clear them up.

(*c*) *Contact Toxicity.*—The contact toxicity results indicate that a surprisingly small number of larvae are prevented by contact with the dressings from completing their development. This, however, is what happens in the field as can be seen, for example, from the result of collecting fully grown larvae leaving a strike which had been treated with dressing No. 1—95 per cent. produced adults.

TABLE 1.—RESULTS OF TESTS WITH BLOWFLY DRESSINGS.

Dressing.	Penetration.	Effect on larvae in strike. All dead after the following period in hours.	Contact toxicity 10 minutes immersion at 30° C. Per cent. mortality.	Irritation.	Protection against oviposition.	Minimum period before restrike (days).	Protection against restrike.	Approximate price per gallon.
B.T.B. 30 ..	Very good	6–12	23	—	+	14	++++	5s. 6d.
B.T.B. 15 ..	Very good	6–12	23	—	±	11	++++	4s. 6d. (2s. home made)
C.B.E. ..	Very good	6–12	25	—	++++	12	++++	12s. 6d.
Commercial No. 1	Very good	0–6	65	++	+	5	++	8s. 6d.
Commercial No. 2	Very good	0–6	47	+	++	5	+	8s. 6d.
Commercial No. 3	Fairly good	12–24	7	+	+	3	+	10s.
Na Pentaborate	Fair	24	25	—	—	2	+	1s. 3d. (home made)
New South Wales Zinc sulphate	Poor	24	50	—	—	1	—	1s. 6d. (home made)
		Water	7					
		Untreated	0					

Effect recorded represented by :
— nil, + slight, ++ definite, ++++ very strong.

(*d*) *Irritation.*—All three commercial dressings were slightly irritant, whereas, the remaining five were quite bland.

(*e*) *Effect on oviposition.*—After application, C.B.E. prevents oviposition on the dressed areas for 24 to 48 hours. B.T.B., on the other hand, is not a good repellent.

(*f*) *Restrike.*—B.T.B. 30 and 15 and C.B.E. protected the dressed area against restrike at least until it had healed, and generally for a considerably longer period. Dressings 1 and 2 gave protection up to five days, but a number of restrikes occurred thereafter. Strikes treated with dressing 3, sodium pentaborate and the zinc sulphate dressing were frequently restruck before they could heal. In these tests 88 struck sheep were used.

(*g*) *Field Strikes.*—A total of 250 field strikes were dressed with the B.T.B. dressings, with the same results as were obtained in the insectary, except that no restrikes occurred.

2. Substitutes for the Tar Oil Fraction.

The tar oil recommended by Lennox (1941) was a straight coal tar fraction (170°–210°C.). It was one of a series supplied by a commercial firm, and was selected as being the most toxic to maggots and least irritant to sheep. When large quantities of this oil were required for the manufacture of B.T.B. it was found that no firms distilled this range on a commercial scale and, although several said they could supply tar oil of this sort, they were eventually unable to do so owing to war-time difficulties. A number of tar and mineral oils and other substances which were available in quantity were, therefore, tested as substitutes. The stability of each dressing, its powers of penetration, and the results of irritation tests are shown in Table 2. The other effect of the tar oil substitute, namely its effect on contact toxicity, is discussed later.

(i) *Other Tar Oils.*

Dressings prepared with various tar oils did not penetrate as well as the original B.T.B. 15, nor were they as stable. In spite of its good reputation as a wetting agent, cresylic acid produced a dressing which penetrated poorly and separated quite rapidly in its container. Commercial dressings H1 and H2 were prepared by spraying crude cresylic acid on to the dry constituents of B.T.B. The dressings obtained on adding water to these moist powders were also unsatisfactory.

Addition of 0·1 per cent. sodium hydroxide to dressings prepared with middle oil 43 and creosote 259 increased both their stability and powers of penetration. Bentonite suspensions are known to be more stable under alkaline than under acid conditions.

A commercial tar oil C (B.P. 170°-300°C.), stated to be similar to the original tar oil, produced a dressing which separated out far more rapidly than the original B.T.B., and penetration was very poor. A ready mixed sample of B.T.B. 30 prepared by the same firm gave good results. However, none of the tar oils tested produced dressings as satisfactory as the original B.T.B.

(ii) *Mineral (Petroleum) Oils.*

Six oils were tested ranging from fly spray kerosene to diesel oil. The three kerosenes produced particularly stable dressings, and diesoline was almost as good. On the other hand, dressings prepared with H200 solvent (190°-210°C.) and diesel oil separated out quite rapidly and to a greater extent than the other four mineral oils. The penetration of these dressings roughly paralleled their stability, the kerosene preparation penetrating the long wool as well as B.T.B. and the short wool almost as well. None of the dressings prepared were irritant, except perhaps that prepared with diesel oil and then only when applied in long wool.

Of the single substances shown in Table 2, power kerosene combined the properties of good penetration and stability with low cost. Tests on thirteen strikes demonstrated that the kerosene dressing had a desirable consistency, penetrated well, was non-irritant, cleaned up strike wounds without excessive scab formation, and prevented restrike.

TABLE 2.—SUBSTITUTES FOR TAR OIL IN B.T.B.

Tar Oil Substitutes. (2 per cent. of final dressing).	Stability.	Irritation.		Penetration*.	
		Short Wool.	Long Wool.	Long 1–1½ in.	Short ½ in.
†Original tar oil	++++	—	—	100	100
†Cresylic acid	+	+	+	60	60
†Crude naphtha	+++	—	+	40	40
†Solvent naphtha	++	—	—	80	75
†Middle oil 43	+	+	+	70	75
†Middle oil 43 + 0·1 per cent. NaOH	++	+	+	75	80
†Creosote 259	+	+	+	65	70
†Creosote 259 + 0·1 per cent. NaOH	++	+	+	70	75
Tar oil C.	+	—	—	40	40
B.T.B. 30 (commercial)	++	—	—	90	95
H1 (B.T.B. 15)	+++	—	+	80	85
H2 (B.T.B. 30)	+++	—	+	85	90
Fly spray kerosene	++++	—	—	90	100
Lighting kerosene	++++	—	—	95	100
Power kerosene	++++	—	—	95	100
H200 solvent	++	—	—	85	95
Diesoline	+++	—	—	70	75
Diesel oil	++	—	+	60	60
50 per cent. creosote 259 + 50 per cent. power kerosene	++++	—	—	120	120
25 per cent. creosote 259 + 75 per cent. power kerosene	+++	—	—	100	100
75 per cent. creosote 259 + 25 per cent. power kerosene	++	—	—	65	70
50 per cent. middle oil 43 + 50 per cent. power kerosene	++++	—	—	120	120
50 per cent. creosote 19§ + 50 per cent. power kerosene	++++	—	—	120	120
50 per cent. creosote 20§ + 50 per cent. power kerosene	+++	—	—	85	90
50 per cent. creosote 24–34† + 50 per cent. power kerosene ..	++++	—	+	70	75
50 per cent. creosote 259 + 50 per cent. solvent naphtha	+	—	++	100	100
50 per cent. tar oil C. + 50 per cent. power kerosene	+++	—	+	95	95
	Poor + Fair ++ Good +++ V. good ++++	Non irritant - Slightly irritant + Definitely irritant ++			

* As an indication of the relative effects of the dressings, figures for penetration were assigned by inspection immediately following the test; the original B.T.B. was taken as 100.
† Supplied by Timbrols Ltd.
§ Supplied by Duratar Ltd.

However it possessed somewhat lower contact toxicity than B.T.B. as shown in Table 3, and it was noticed that, although the dressing cleaned up the strike wound, the larvae did not appear to be much irritated

TABLE 3.—CONTACT TOXICITY RESULTS.

Treatment.	Percentage mortality after immersion for—		
	5 Minutes.	10 Minutes.	15 Minutes
B.T.B. original	30	45	80
Power kerosene dressing	15	25	55
Water	5	17	10

by it. Those that remained in the dressed area and continued to feed succumbed to boric acid poisoning; others fell off, whereas others again crawled away into adjacent undressed areas and in five out of thirteen strikes managed to establish small pockets of larvae. In assessing the significance of these results the immediate and important consideration is that the sheep should be rid of maggots following dressing.

(iii) *Mixtures of Tar Oils and Mineral Oils.*

As the kerosene dressing was unsatisfactory because of its low contact toxicity, and the tar oil dressings undesirable on account of their comparatively poor powers of penetration and their tendency to produce irritation, a number of mixtures were tested consisting of kerosene and tar oils, and one mixture of creosote and solvent naphtha. Replacing the 2 per cent. tar oil in B.T.B. with a 50:50 mixture of creosote 259 and power kerosene produced a non-irritant dressing, as stable as B.T.B., which penetrated long and short wool more readily than any other dressing tested in this series. On mixing the two oils a slight sludge settled out. This was probably a colloidal pitch and was discarded, but can be retained, without reducing the efficiency of the dressing. Departure from the 50:50 mixture in either direction resulted in lessened stability and poorer penetration. Thus the mixture containing 75 per cent. power kerosene behaved largely as if the creosote were not present, and the mixture containing 25 per cent. kerosene as if the kerosene were absent. A mixture of middle oil and kerosene behaved in the same way as the 50:50 creosote-kerosene mixture. Tests with three additional creosotes of known distillation range showed that the penetration of the resulting dressing varied with the creosote used, but could not be correlated with their distillation range, nor with their tar acid content. These are the only data normally available for tar oil samples. A 50:50 mixture of kerosene and tar oil C (mentioned above) also produced a markedly more satisfactory dressing than did the tar oil alone. An interesting feature of these results was the excellent penetration of the dressing prepared with a 50:50 mixture of creosote and solvent naphtha, for either of these alone produced dressings which penetrated poorly. It is interesting also that the stability of this dressing is poor, for this shows that there

is no necessary relationship between stability and rapid penetration. A close correlation had been suggested by the results with mineral and tar oils.

Owing to the ready availability of large quantities of the two types of tar oil fraction and their low cost (cresosote 1s., middle oil 1s. 6d. a gallon), insectary and field tests were carried out with 50:50 mixtures with kerosene. More attention was paid initially to creosote 259, as it was then believed that, being a standardized product, it would vary less than middle oil. It was later discovered, however, that the standards laid down for wood preservatives allow considerable variation. Six samples of creosote 259 and 4 samples of middle oil 43 were obtained from different distillations by the same firm. Taking penetration as the criterion and using 50:50 mixtures with kerosene, the best of the creosote samples were slightly better than any of the middle oil samples, but the worst was considerably less efficient than any of the middle oils tested and indeed would be rated at 90 in Table 2. From its narrower distillation range 200°–240°C. (cf. creosote 240°–330°C. approx.), middle oil 43 might well be expected to be a more uniform product than creosote 259. The slight differences revealed by analyses of the various batches are principally due to differences in the raw materials used. The tar distillers naturally are not able to guard against this in their various batches. The stability of the various dressings prepared with the samples of creosote and middle oil was very similar.

During the tests with tar oil substitutes it was confirmed that addition of the tar oil to the dressing when mixed to a thin paste with water was more satisfactory than after all the water had been added. If some of the creosote samples were added after all the water had been mixed in, much of the tar oil remained on the surface for several hours after initial mixing. Stirring or shaking several hours later resulted in almost all the droplets going into suspension, with attendant increase in powers of penetration of the dressing. As a general rule the tar oils have been found to mix in progressively less easily as more water is added after the paste stage.

In the spring of 1941, 122 field strikes were dressed with the 50:50 creosote kerosene dressing. Five sheep were restruck within 14 days of dressing, and three of these restrikes occurred on areas which had become septic, a condition favoured by the deeply eroded nature of the wound. The season was a favourable one for flies, approximately 25 per cent. of the flock being struck during the test period. As a result of these tests the 50:50 mixtures of kerosene with creosote 259 or middle oil 43 were recommended (Anon. 1941) as substitutes for the tar oil fraction. Dressings prepared with these substitutes have been widely used throughout Australia for the past 18 months. More recently a modification of this dressing with improved contact toxicity has been discovered, but this can best be described following the results of contact toxicity tests.

The effect of complete immersion of fully fed larvae for both 5 and 10 minutes in each dressing was examined. All the dressings were not tested at the same time, and since it was desirable to be able to compare the contact toxicity of one with another, at least in a general way, each dressing was given a rating. This rating was obtained by

subtracting from the average percentage mortality following immersion in the dressing in question, the average percentage mortality following similar treatment in the original B.T.B. over the same series of tests. A high minus rating, therefore, indicates poor contact toxicity. The results for 5-minute dips are shown in Table 4, each figure being based on tests with from 80 to 200 larvae, using 20 larvae for each test.

TABLE 4.—CONTACT TOXICITY OF DRESSINGS TO MATURE 3RD INSTAR LARVAE (5-MINUTE DIPS AT 30°C.)

Tar Oil Substrate.	Rating.		
	− 20 to − 29.	− 30 to − 39.	− 40 and over.
Cresylic acid	..	+	..
Crude naphtha	..	+	..
Solvent naphtha	..	+	..
Middle oil 43	..	..	+
Middle oil 43 + 0·1 per cent. NaOH	..	..	+
Creosote 259	..	..	+
Creosote 259 + 0·1 per cent. NaOH	..	..	+
Kerosene—Fly spray	+	..	..
Kerosene—Lighting	..	..	+
Kerosene—Power	..	+	..
H200 solvent	..	..	+
Diesoline	..	..	+
Diesel oil	..	+	..
50 per cent. creosote 259 + 50 per cent. power kerosene	+	..	..
25 per cent. creosote 259 + 75 per cent. power kerosene	..	+	..
75 per cent. creosote 259 + 25 per cent. power kerosene	..	+	..
50 per cent. middle oil 43 + 50 per cent. power kerosene	..	..	+
50 per cent. creosote 19 + 50 per cent. power kerosene	..	..	+
50 per cent. creosote 24–34 + 50 per cent. power kerosene	..	+	..
50 per cent. creosote 259 + 50 per cent. solvent naphtha	+	..	..
50 per cent. crude naphtha + 50 per cent. power kerosene	..	..	+

No differences were noted when four samples of both creosote 259 and middle oil 43, each from different distillations, were tested for contact toxicity. It is a noticeable feature that all these dressings are considerably inferior in contact toxicity to B.T.B. containing the original tar oil. In this series of tests the original B.T.B. allowed 45 per cent. emergence, and 92 per cent. of all controls emerged. Table 5 gives the averages of 8 contact toxicity tests with the 4 more important dressings in this series. Dressings containing 2 per cent. creosote 259, or 2 per cent. middle oil 43, were not tested extensively because they had some undesirable properties.

These figures show that the creosote–kerosene mixture is more toxic than power kerosene alone. The different figures for mortality after immersion in the original B.T.B., which are used in Tables 1, 4, and 5, are a result of the variations in resistance of fully-fed larvae which may occur from one series of experiments to the next.

TABLE 5.—CONTACT TOXICITY OF FOUR DRESSINGS. FIVE MINUTES IMMERSION.

Tar Oil Substitute.	Percentage Mortality.
Original tar oil	62
50 : 50 Creosote 259 + power kerosene	49
50 : 50 middle oil 43 + power kerosene	34
Power kerosene	29
Control	4

Good contact toxicity is generally associated with great irritant effect on the larvae, and this acts as a compensating factor to reduce the mortality of larvae when strikes are dressed. Thus dressings with good contact toxicity often cause the majority of larvae to leave the wound in the first few minutes, while less effective mixtures may not drive them out for a much longer period. The mortalities caused by contact with the dressings are thus often more similar under field conditions than when dip tests are done and, in general, are very low for fully grown larvae.

(iv) *Eucalyptus and Other Essential Oils.*

In view of the evidence (Waterhouse, unpublished) that the struck sheep is the principal breeding ground of *L. cuprina,* it was considered that an increase in the contact toxicity of B.T.B. was desirable. Additional substances considered as possible contact agents were, therefore, tested. Table 6 gives the results of tests with a series of commercially available essential oils and their emulsions. Even the pure

TABLE 6.—PERCENTAGE MORTALITY AFTER IMMERSION AT 25°C. 40 LARVAE WERE USED FOR EACH PERCENTAGE.

Oils (Undiluted).	5 Minutes Immersion.	10 Minutes Immersion.	Emulsions.	5 Minutes Immersion.	10 Minutes Immersion.
Eucalyptus dives var. A. ..	43	55	*E. dives** var. A.	7	10
E. dives var. C. ..	10	43	*E. dives*† var. C.	10	35
E. polybractea Residue ..	5	20	*E. polybractea*† Res. ..	5	10
E. phellandra ..	25	47	*E. phellandra** ..	7	10
E. phellandra B.	17	20	*E. phellandra** B.	12	7
Medicinal eucalyptus oil ..	30	65	2 per cent. Gardinol (emulsifier)	2	0
Camphor oil ..	57	57	Water	7	10
Titrol	32	45	Control (untreated)	2	12

* 2 g. Gardinol + 100 ml. H_2O + 15 ml. essential oil.

† 1 g. Gardinol + 100 ml. H_2O + 15 ml. essential oil.

Most of these oils were supplied by Mr. M. R. Freney.

oils did not produce a very high mortality and, therefore, compare unfavourably with creosote 259, middle oil 43, and solvent naphtha, which cause 100 per cent mortality in 5-minute dips.

(v) *Mixtures Containing Carbon Tetrachloride and Ortho-dichlorbenzene.*

A series of contact toxicity tests were performed with ortho-dichlorbenzene which is produced in Australia in excess of local demands. The results are given in Table 7. It can be seen that all larvae were killed by B.T.B. containing 2 per cent. ortho-dichlorbenzene (ODB), but that, with 1 per cent., more than half the larvae survived.

TABLE 7.—CONTACT TOXICITY TESTS WITH ORTHO-DICHLORBENZENE.

Contact Agent.	Percentage Mortality After Immersion for—		
	½ Minute.	5 Minutes.	10 Minutes.
B.T.B. with 1 per cent. ODB	..	100	100
B.T.B. with 1 per cent. ODB + 1 per cent. kerosene	..	40	35
50 per cent. ODB in kerosene	100	..	..
25 per cent. ODB in kerosene	100	..	..
10 per cent. ODB in kerosene	100	..	..
5 per cent. ODB in kerosene	82	..	..
Control (water)	..	2	2

Replacing the tar oil with ODB, however, produces a dressing which separates out very rapidly and which penetrates poorly. A 50 : 50 mixture of ODB with kerosene produces a more stable dressing, but the powers of penetration were slightly worse than the ODB alone and the contact toxicity was not appreciably better than B.T.B. containing creosote 259 and kerosene. Included in this series of tests was a dressing recommended by Hobson (1941) and containing:—

Lysol dressing—

Lysol	.. 5 per cent.	Mix and add the water.
Carbon tetrachloride	.. 4 per cent.	
Lighting kerosene	.. 11 per cent.	
Water ..	.. 80 per cent.	

This produced 100 per cent. mortality after 5- and 10-minute dips. It is a stable emulsion, which penetrates struck fleece fairly well, but does not go into dry wool with nearly the same ease as B.T.B. This dressing was considered unsuitable because it produced slight irritation and more particularly because it contained no stomach poison to protect against restrike. A further serious disadvantage under war-time conditions is that the dressing contains an appreciable quantity of carbon tetrachloride which is in short supply. When the dressing was prepared with either ortho-dichlorbenzene or with creosote 259 in place of carbon tetrachloride, satisfactory emulsions also resulted.

A series of B.T.B. dressings were next prepared with the non-water constituents of the dressings just discussed substituted for the tar oil. Contact toxicity tests are shown in Table 8. Although there did not appear to be much difference in toxicity between the lysol-carbon tetrachloride-kerosene dressing and the modification with ortho-dichlorbenzene in place of carbon tetrachloride, the creosote 259 modification produced very low mortality.

B.T.B. prepared with the mixtures of contact agents (referred to as A and B in Table 8) showed disappointingly low mortality when 2 per cent. was incorporated, but high mortality when 10 per cent. or 20 per cent. was used. At 20 per cent. concentration contact mixture B proved more effective than contact mixture A and produced 100 per cent. mortality even when the period of immersion was reduced to half a minute. The 50:50 kerosene–creosote mixture caused uniformly low mortality, and mixtures of kerosene and ortho-dichlorbenzene as tar oil substitute were not as promising as when lysol also was present. If an equal quantity of linseed soap was substituted for the lysol (Contact C) a less effective contact agent was produced.

TABLE 8.—CONTACT TOXICITY TESTS WITH VARIOUS DRESSINGS.

Dressing.	Percentage Mortality After Immersion at 30°C. for—			
	5 Minutes.	2 Minutes.	1 Minute.	½ Minute.
Control (water)	2	3	6	2
B.T.B. 15 (original)	79	52	54	34
Lysol dressing	92	..	..	..
B.T.B. with 2 per cent. Contact A ..	28	..	..	..
B.T.B. with 5 per cent. Contact A ..	17	..	..	..
B.T.B. with 10 per cent. Contact A..	73	76	..	..
B.T.B. with 20 per cent. Contact A..	90	97	100	89
Lysol dressing with ODB	87	..	..	..
B.T.B. with 2 per cent. Contact B ..	17	..	..	..
B.T.B. with 10 per cent. Contact B..	93	89	..	..
B.T.B. with 20 per cent. Contact B (B.K.B.)	100	100	100	100
Lysol dressing with creosote 259 ..	10	..	..	..
B.T.B. with 2 per cent. ODB + 2 per cent. power kerosene	37	21	..	..
B.T.B. with 4 per cent. ODB + 4 per cent. power kerosene	80	51	..	..
B.T.B. with 5 per cent. ODB + 5 per cent. power kerosene	81	..	..	..
B.T.B. with 5 per cent. ODB ..	95	..	..	..
B.T.B. with 1 per cent. creosote 259 + 1 per cent. power kerosene ..	5	3	12	5
B.T.B. with 20 per cent. Contact C. ..	..	66	51	90

Contact A—
- CCl_4 .. .. 20 per cent.
- Lysol .. .. 25 „
- Kerosene .. .. 55 „

Contact B.—
- Ortho-dichlorbenzene .. .. 20 „
- Lysol .. .. 25 „
- Kerosene .. .. 55 „

Contact C.—
- Ortho-dichlorbenzene .. .. 20 „
- Linseed soap .. .. 25 „
- Kerosene .. .. 55 „

It was a noticeable feature of B.T.B. dressings containing contact mixtures A and B that an increase in the amount of contact agent resulted in greater stability (Table 9.)

Table 9.—Effect of Proportion of Contact Agent on Stability of Dressing.

Dressing.	Percentage Clear Solution. 24 hours After Preparation.
B.T.B. with 2 per cent. Contact A.	55
B.T.B. with 5 per cent. Contact A.	50
B.T.B. with 10 per cent. Contact A.	10
B.T.B. with 20 per cent. Contact A.	5

With the greater amount of contact agent the dressing appeared to flocculate slightly, but has always shown appreciably less tendency to settle out over long periods than both the original B.T.B. and the creosote-kerosene modification.

For this series the power of penetration of each dressing follows a similar trend to its stability; mixtures containing less than 5 per cent. of the contact agent do not penetrate quite as well as the creosote-kerosene dressing, but above this the penetration is at least as good. Moreover it has been found that dressings containing 20 per cent. of contact mixture B have consistently good powers of penetration, whereas, as pointed out earlier, the penetration of dressings containing creosote 259 or middle oil 43 varies quite widely from one batch of tar oil to the next. This uniformity is probably due to the fact that the constituents of mixture B are far more constant in composition than are different batches of tar oils.

No irritation was noted in long or short wool when any of these dressings were tested. The dressing containing 20 per cent. of mixture B was next tested against 35 field strikes. Immediately a strike was dressed, larvae started to drop off, but a noticeable feature of this dressing, compared with the original B.T.B., was the large number of larvae which were immobilized when half out of the fleece. Many dead, but no live larvae, could be seen next day. The strikes all healed satisfactorily. The larvae dropping off two strikes dressed with B.T.B. containing 10 per cent. of mixture B and three strikes dressed with B.T.B. containing 20 per cent. were collected and placed in dry sand. Of the former, 55 per cent. and of the latter 3 per cent. emerged. It is clear then that the dressing containing 20 per cent. mixture B possesses far better contact toxicity and better stability than the original B.T.B. or the creosote-kerosene modification, and greater uniformity than the latter at least. It is now referred to as B.K.B., and its use under practical conditions has been recommended.

If the present recommendation that B.T.B. be sold in powder state is retained, B.K.B. possesses the slight disadvantage that the bottle containing the tar oil substitute is much larger, and when packed in its carton is protected by a thinner layer of powder. In addition, there is an increase in cost of approximately 1s. 3d. a gallon.

The composition of B.K.B., recorded as percentage by weight, is:

Boric acid		15·0
Lysol	5·3	20·0
Ortho-dichlorbenzene	5·4	
Kerosene	9·3	
Bentonite		3·0
Agral 2		0·5
Water		61·5

A dressing of high contact toxicity, stability, and powers of penetration, with no irritant effect, can also be prepared containing 25 per cent. of contact mixture B.

3. Tests with Bentonites.

A number of bentonites and so-called bentonites from American and Australian sources have been tested from time to time for suitability in B.T.B. With each sample a bentonite rating test (Table 10) was carried out according to the method described by Fyfe (1934).

To 4 g. of bentonite, dried at 100°C., 5 g. of magnesium oxide was added, then 100 ml. distilled water. This mixture was placed in a stoppered bottle, shaken for one hour, and then allowed to stand for 24 hours. A good bentonite gives a fairly stiff gel which will not break on inverting the container. The percentage of supernatant clear liquid subtracted from 100 gives the bentonite rating, anything having a value greater than 50 being accepted as a bentonite, whereas ordinary clay gives a value of 10 or less.

TABLE 10.—RATINGS OF AMERICAN AND AUSTRALIAN BENTONITES.

Bentonite.	Rating.
Powdered American bentonite A.	69
Powdered American bentonite B.	100
Aquaseal, Volclay, Kwiksol } granular	100
Wyomacla	100
Wilkinite	72
Western Australia—Commercial No. 1	66
Western Australia—Commercial No. 2	69
Western Australia—Marchagee	47
Western Australia—Mumballup	53
Western Australia—Collie	49
Western Australia—Jennacubbine	43
New South Wales sample (Gunnedah)	26

All except two of the American bentonites gave a 100 per cent. rating. One exception was a bentonite which was imported from the U.S.A., but ground locally. It is clear that the processing that the American samples are put through improves their gelling powers considerably.

Bentonites from Australian sources, and not chemically processed, had a maximum rating of 69. Approximately half of the Australian samples tested could not be classified as bentonites.

There is a good correlation between high bentonite rating and high stability when used as a constituent of B.T.B. In general, the more stable dressings penetrated better than the less stable. For instance, B.T.B. prepared with the locally-ground American bentonite was not stable and separated out rapidly. Even immediately after shaking, the dressing did not penetrate the fleece nearly as well as the original dressing. This suggests that one of the roles which bentonite performs in the dressing is that of increasing the powers of penetration, even if it does so indirectly, perhaps, by affecting the nature and stability of the emulsion. This is not the whole story, however, for the dressing prepared with bentonite from Collie, W.A., penetrated very well, although it settled out very rapidly after shaking.

4. Discussion.

The tests detailed in the foregoing sections form a good illustration of the difficulty of predicting the effect on contact toxicity, powers of penetration, and stability of B.T.B. when substitutes are used in place of the original tar oil or bentonite.

The contact component of the dressing exerts a marked effect on stability and, in turn, rapid penetration into the fleece is almost always associated with good stability. A poor bentonite can markedly lower the powers of penetration of an otherwise excellent dressing. It must be noted, however, that instability due to the contact agent (2 per cent. of 50 : 50 creosote 259 + solvent naphtha) or to the bentonite (Collie, W.A., sample) does not always result in a lessening of the rapidity, or ease, of penetration.

By replacing the tar oil fraction with certain other substances and using these at a much higher concentration than the tar oil, it is possible to improve the contact toxicity of B.T.B. very greatly. The B.K.B. dressing containing 20 per cent. of a mixture of ortho-dichlorbenzene, lysol, and kerosene kills larvae rapidly in the dressed area and most of those that leave. If 25 per cent. of the mixture were used, a still smaller percentage of larvae should survive. If, however, all larvae remaining on the sheep are killed with the 20 per cent. dressing, it is questionable whether the additional cost of the 25 per cent. mixture is justified, since large numbers of fully grown larvae are allowed to escape unharmed when the wool is shorn down as close as possible over the struck area before the dressing is applied.

It is interesting to examine the effect of killing all larvae in strike wounds as a means of lowering the *L. cuprina* population. There is good evidence that the *L. cuprina* population is dependent largely upon flies produced from struck sheep, since few *L. cuprina* were found to breed in carcases (Waterhouse, unpublished). Therefore it appears that the *L. cuprina* population can be readily influenced by:

(*a*) Reducing the susceptibility of sheep (by breeding, Mules operation, controlled tailing, &c.);

(*b*) Killing all larvae in strike wounds.

It has been suggested (J.B.C. 1940) that strikes could be cleaned up over a large piece of strong canvas and the struck wool containing maggots destroyed. This is not a very practicable method under field conditions. On the other hand it would, perhaps, be possible to leave about 1 inch of wool over the strike, thereby retaining most of the maggots in the wound, and then to apply a dressing which would kill them. This is contrasted with the usual practice of shearing the wool as close to the skin as possible and then scraping the maggots out with the shears. Most of the large maggots are able to produce flies when treated in this fashion. The main objection to the suggested procedure is that it is more difficult to trace extensions of the strike wound, unless all the wool is removed.

5. Acknowledgments.

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The Influence of Dissolved Tin on the Growth of *Clostridium botulinum* in Canned Vegetables.

1. Experiments with Beetroot and Carrots.

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Summary.

Several strains of *Cl. botulinum* were inoculated into cans of beetroot and carrots. There was regular growth and toxin production if the cans had been lacquered internally, but not when unlacquered cans were used. The inhibition of growth in unlacquered cans was associated with concentrations of dissolved tin which consistently exceeded the values for lacquered cans.

The addition of a soluble tin-citrate complex to each vegetable produced inhibition of growth, both in lacquered cans and in test tubes. The concentrations of tin required to prevent growth were approximately 150 p.p.m. in beetroot and 30–60 p.p.m. in carrots. This bacteriostatic effect of dissolved tin affords an explanation of the inhibition observed in unlacquered cans.

1. Introduction.

During the course of studies on the growth of *Cl. botulinum* in canned vegetables, it was observed that the organisms failed to grow in some vegetables processed in plain unlacquered‡ cans, but growth occurred regularly when internally lacquered‡ cans were used. In investigating this phenomenon, determinations of copper, iron, and tin were made in beetroot processed in lacquered and in plain cans. Although only small differences were apparent in the levels of copper and iron, the concentration of tin in the contents of plain cans greatly exceeded that in lacquered cans. This paper deals with experiments in beetroot and carrots and demonstrates that the inhibition of growth which occurs in plain cans is due to the bacteriostatic effect of dissolved tin.

Few references to the anti-bacterial activity of tin are available. Hotchkiss (1923) found stannic chloride in peptone-water inhibitory to *E. coli*, but owing to precipitation with the peptone the effective concentration was unknown. Reitler and Marburg (1943) report a mild bactericidal action against various non-sporing pathogenic bacteria suspended in Ringer's solution together with spongy metallic tin.

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‡ Vegetables are processed commercially in tinplate containers which are frequently protected internally by lacquers.

2. Methods.

All inoculations were made in No. $2\frac{1}{2}$ cans (capacity approximately 850 ml.) which had been packed and processed in these laboratories according to the schedules recommended by the National Canners Association (1942). Lacquered cans were protected internally by a double coating of an oleo-resinous lacquer which was either of an acid-resistant, citrus, or sulphur-resistant type. Prior to inoculation, cans were steamed for at least 30 minutes, and the inoculum was introduced aseptically through a small puncture made in the can end. At the time of inoculation portion of the contents was withdrawn for the determination of pH, and, when required, the tin solutions were introduced. Punctures were closed by soldering while the cans were still hot.

The inoculum consisted only of spores which were harvested from Robertson's cooked meat medium. Spore suspensions were heated at 80°C. for 10 minutes and stored at 1°C. Where specified the size of inoculum refers to the number of viable spores.

Inoculated cans were incubated either at 30° or 37°C. They were examined daily and any swelled cans were opened immediately. The usual aseptic techniques of the official A.O.A.C. methods (1940) were observed. Immediately after opening, the contents of each can were examined microscopically and the pH determined. Material for chemical analysis was transferred aseptically to pyrex flasks and autoclaved for 60 minutes at 120°C. before being analysed. Tin was determined by the method of Morris and Bryan (1931) and iron and copper by the methods of McCance, Widdowson, and Shackleton (1936).

Tin solutions were prepared from reagent-quality chemicals and glass-distilled water. Tin was added as a soluble citrate complex prepared by dissolving one mole of stannous chloride in two moles of sodium citrate solution. (A stock solution of $0 \cdot 5$ M $SnCl_2$ in $1 \cdot 0$ M sodium citrate was diluted as required.) In some early experiments tin was added as stannous chloride or in the form of stanno-oxalate or stanno-tartrate complexes.

For experiments in test tubes, media were prepared by infusing the finely diced vegetable in an equal weight of 1 per cent. sodium chloride in glass-distilled water, and dispensing in tubes one part of solid and two parts of liquid. With beetroot, $0 \cdot 2$ per cent. glucose was added to stimulate growth. Tin solutions were added prior to autoclaving the tubes, which were subsequently inoculated with spores and incubated anaerobically in a McIntosh and Fildes jar with 5 per cent. carbon dioxide in the atmosphere.

3. Results.

Eight strains of *Cl. botulinum* have been used in these experiments. Four type B strains 968, 1787, A12, and L12, and two type A strains L4 and Q7 had been isolated recently in Australia. Two strains, U.S. type A and U.S. type B originally obtained from America, had been maintained in this country for some years. *Cl. sporogenes* N.C.A. strain No. 3679 and another proteolytic *Clostridium* designated A40 were also used in some of the experiments.

Cans were judged to be spoiled when microscopic examination of the liquor revealed an abundance of organisms, in contrast to unspoiled cans which showed the virtual absence of bacteria. The contents of spoiled cans were characterized by an off-odour discernible to experienced, but not always to inexperienced, observers. The liquors from cans spoiled by *Cl. botulinum* were toxic when fed to guinea pigs, whereas the liquors from unspoiled cans produced no ill effects.

(i) *Experiments with Beetroot.*

(*a*) *Inoculation of plain and lacquered cans.*—Table 1 shows the details for a representative selection of the cans inoculated. No tin was added to these cans. Of the 25 cans inoculated 12 were spoiled. From all unspoiled cans viable organisms were recovered. In five unspoiled cans (Table 1, Nos. 8, 9, 11, 12, and 15) the inoculum was recovered in approximately the same numbers as seeded, both before and after heating at 80°C. for 10 minutes. In these cans, therefore, no germination of spores had occurred, and the viability of the original inoculum was substantially unimpaired.

Of the twelve spoiled cans, seven were flat and five were swelled. Swells developed only when *Cl. botulinum* had grown in cans with a relatively high initial pH.

TABLE 1.—INOCULATION OF BEETROOT IN PLAIN AND LACQUERED CANS.

Can No.	Internal Lacquer.	Inoculum. Strain.	Inoculum. No. of Spores.	Initial pH.	Incubation Days at 37° C.	Condition of Can at Opening.	Spoilage.	Final pH.	Metals Content of Liquor p.p.m. Sn.	Fe.	Cu.
1	Yes	A40	$1 \cdot 6 \times 10^6$	5·71	27	Flat	Yes	5·29	16	..	..
2	Yes	968	$4 \cdot 0 \times 10^8$	5·71	6	Swell	Yes	5·53	..	..	..
3	Yes	1787	$1 \cdot 7 \times 10^8$	5·71	6	Swell	Yes	5·32	..	..	..
4	Yes	A40	$1 \cdot 6 \times 10^6$	5·10	27	Flat	Yes	5·07	11	..	..
5	Yes	968	$4 \cdot 0 \times 10^8$	5·10	27	Flat	Yes	5·12	17	..	..
6	Yes	1787	$1 \cdot 7 \times 10^8$	5·10	27	Flat	Yes	5·30	15	..	..
7	No	A40	$1 \cdot 6 \times 10^6$	5·22	27	Flat	No	5·09	289	..	..
8	No	968	$4 \cdot 0 \times 10^8$(*)	5·22	27	Flat	No	5·28	290	..	..
9	No	1787	$1 \cdot 7 \times 10^8$(*)	5·22	27	Flat	No	5·19	308	..	..
10	No	A40	$1 \cdot 6 \times 10^6$	5·77	27	Flat	No	5·30	315	..	..
11	No	968	$4 \cdot 0 \times 10^8$(*)	5·77	20	Flat	No	5·52	278	..	..
12	No	1787	$1 \cdot 7 \times 10^8$(*)	5·77	20	Flat	No	5·50	232	..	..
13	Yes	968	$4 \cdot 0 \times 10^8$	5·71	5	Swell	Yes	5·67	6	11	10
14	Yes	1787	$1 \cdot 7 \times 10^8$	5·71	5	Swell	Yes	5·79	10	4	9
15	No	968	$4 \cdot 0 \times 10^8$(*)	5·71	5	Flat	No	5·86	156	17	7
16	No	1787	$1 \cdot 7 \times 10^8$	5·71	5	Flat	Yes	5·93	126	14	6
17	Yes	U.S. Type A	..	5·71	4	Swell	Yes	5·54	50	..	..
18	Yes	U.S. Type A	..	5·10	7	Flat	Yes	5·33	18	..	..
19	No	U.S. Type A	..	5·71	7	Flat	No	5·50	167	..	..
20	No	U.S. Type A	..	5·22	7	Flat	No	5·33	179	..	..
21	No	U.S. Type B	..	..	65	Flat	No	5·50	386	..	..
22	No	968	..	..	65	Flat	No	5·39	344	..	..
23	No	1787	..	..	65	Flat	No	5·24	384	..	..
24†	Yes	3679	..	5·37	30	Flat	Yes	4·99	9	..	..
25†	No	3679	..	5·80	30	Flat	No	5·57	246	..	..

* Quantitative determinations demonstrated approximately this number of spores to be present when the can was opened.

† Cans incubated at 30° C.

The tin analyses recorded in Table 1 indicate a marked difference between the contents of lacquered and plain cans. The higher contents of dissolved tin in plain cans are associated with failure of the inoculated organisms to grow. Thus spoilage occurred in all lacquered cans, but only in one plain can (No. 16). This can had the lowest content of dissolved tin of all the plain cans.

(*b*) *Inoculation of lacquered cans containing added tin.*—Preliminary experiments showed that when stannous chloride, or stannous chloride dissolved in Rochelle salt solution, was added to lacquered cans to produce a tin concentration of 200 to 250 p.p.m., no growth of strains 968 and 1787 occurred. The results obtained when varying amounts of tin were added as tin-citrate complex to lacquered cans of beetroot are shown in Table 2.

TABLE 2.—INOCULATION OF BEETROOT IN LACQUERED CANS WITH TIN ADDED.

Concentration of Added Tin p.p.m.	Inoculum 968.			Inoculum L4.		
	Initial pH.	Final pH.	Spoilage.	Initial pH.	Final pH.	Spoilage.
0	5·31	5·12	Yes	5·36	5·19	Yes
90	5·38	5·05	No	5·17	5·21	Yes
120	5·39	5·05	No	5·03	5·07	No
150	5·31	5·08	No	5·37	5·07	No
180	5·34	5·08	No	5·33	5·07	No

Cans incubated at 30° C. for 30 days.

(*c*) *Experiments in test tubes.*—Table 3 shows the results obtained when test tubes containing beetroot media were inoculated with six strains of *Cl. botulinum.*

TABLE 3.—THE EFFECT OF ADDED TIN ON *Cl. botulinum* INOCULATED INTO BEETROOT MEDIUM.

Concentration of Added Tin p.p.m.	Initial pH.	Inoculum.					
		968.	1787.	L12	A12.	Q7.	L4.
0 ..	5·50	+ (3)	+ (3)	+ (3)	+ (3)	+ (3)	+ (3)
90 ..	5·38	+ (3–6)	+ (3–6)	+ (3–6)	+ (3–6)	+ (3–6)	+ (3–6)
120 ..	5·32	+ (25)	+ (19)	+ (19)	+ (18)	+ (8)	+ (20)
150 ..	5·42	—	—	—	—	—	—
180 ..	5·35	—	—	—	—	—	—

+ Growth observed after ( ) days at 37° C.
— No growth after 32 days at 37° C.

Growth was indicated by the production of gas and was confirmed microscopically. The addition of tin in concentrations of 150 p.p.m. and higher has inhibited growth of all strains for at least 32 days,

whereas in the absence of added tin strong growth occurred within three days. Intermediate concentrations delayed the time at which evidence of growth became apparent.

(ii) *Experiments with Carrots.*

(*a*) *Inoculation of plain and lacquered cans.*

TABLE 4.—INOCULATION OF CARROTS IN PLAIN AND LACQUERED CANS.

Can No.	Internal Lacquer.	Inoculum.	Initial pH.	Incubation Days 37° C.	Condition of Can at Opening.	Spoilage.	Final pH.	Tin in Liquor. p.p.m.
1	Yes	968	5·12	12	Swell	Yes	5·23	16
2	Yes	968	5·27	5	Swell	Yes	5·64	18
3	Yes	L4	5·02	6	Swell	Yes	5·48	22
4	Yes	1787	5·02	7	Swell	Yes	5·51	27
5	No	968	5·20	15	Flat	No	4·90	44
6	No	968	5·26	15	Flat	No	4·90	50
7	No	L4	5·02	21	Flat	No	5·00	59
8	No	1787	4·99	21	Flat	No	4·99	72
9	Yes	3679	5·05	8	Swell	Yes	5·16	33
10	No	3679	5·02	21	Flat	No	5·02	59

Table 4 shows the results for ten cans of carrots inoculated with three strains of *Cl. botulinum* and *Cl. sporogenes* No. 3679. All the lacquered cans swelled and were spoiled, but none of the plain cans was spoiled after incubation for 15 and 21 days. The contents of the plain cans have dissolved a consistently greater amount of tin than the lacquered cans.

(*b*) *Inoculation of lacquered cans with added tin.*—Table 5 shows the results with a series of lacquered cans to which had been added varying amounts of tin in the form of tin-citrate complex.

TABLE 5.—INOCULATION OF CARROTS IN LACQUERED CANS WITH TIN ADDED.

Concentration of Added Tin p.p.m.	Inoculum L4.			Inoculum 968.		
	Initial pH.	Final pH.	Spoilage.	Initial pH.	Final pH.	Spoilage
0	5·19	5·17	Yes*	5·15	5·17	Yes†
15	5·15	4·99	No	5·19	5·09	Yes
30	5·16	5·02	Yes	5·18	4·99	No
60	5·16	5·00	No	5·18	5·02	No
90	5·14	4·99	No	5·16	4·98	No

Cans Incubated at 30° C. for 29 days.

* Swelled after 17 days.

† Swelled after 26 da

(*c*) *Experiments in test tubes.*—Results for six strains of *Cl. botulinum* inoculated into carrot medium in test tubes are shown in Table 6.

TABLE 6.—THE EFFECT OF ADDED TIN ON *Cl. botulinum* INOCULATED INTO CARROT MEDIUM.

Concentration of Added Tin p.p.m.	Initial pH.	Inoculum.					
		968.	1787.	L12.	A12.	Q7.	L4.
0	5·27	+ (9)	+ (9)	+ (9)	+ (9)	+ (19)	+ (9)
30	5·27	+ (12)	+ (12)	+ (12)	+ (12)	+ (21)	—
60	5·23	—	—	—	—	+ (19)	—
90	5·20	—	—	—	—	—	—
120	5·17	—	—	—	—	—	—

\+ Growth after ( ) days at 37° C.
— No growth after 26 days at 37° C.

4. Discussion.

From the above results it may be concluded that the failure of *Cl. botulinum* to grow in beetroot and carrots packed in plain cans is associated with higher levels of dissolved tin than are found in internally lacquered cans in which growth occurs regularly. The concentrations of dissolved iron and copper were not appreciably greater in the plain cans. Moreover, growth in lacquered cans is inhibited by adding dissolved tin in concentrations similar to those found in plain cans.

In both vegetables, growth in test tubes may be inhibited by the addition of a soluble tin-citrate complex. The concentrations required to inhibit growth in test tubes are comparable with the concentrations found inhibitory when tin is added to lacquered cans, and are of the order of 150 p.p.m. in beetroot and 30–60 p.p.m. in carrots.

This bacteriostatic influence of tin is sufficient to explain the inhibition of growth which has been observed in plain cans of these two vegetables, in which the concentrations of naturally dissolved tin have been generally in excess of the above critical values. Growth has been observed in only one plain can of beetroot, which had a tin content of 126 p.p.m.

Earlier reports on the growth and toxin production of *Cl. botulinum* in canned vegetables mention irregular development of the organisms in several products, including beetroot. Schoenholz, Esty, and Meyer (1923) inoculated 31 cans of beets with large numbers of detoxified spores, and after incubation for periods up to twelve months found swells in only 17. The contents from ten of the flat cans were non-toxic but contained *Cl. botulinum* organisms. There is no indication whether the cans used were plain or lacquered internally, and inhibition by dissolved tin may have been responsible for the ten cans remaining non-toxic.

Although the experiments reported here show that certain concentrations of dissolved tin have been adequate to prevent the growth of *Cl. botulinum* for limited periods of incubation, it is not known whether these concentrations would suffice for more extended incubation. Further, the influences of such factors as temperature of incubation, pH, size of inoculum, and variations in batches of vegetables are yet to be studied. However, inhibition in plain cans is not likely to be cancelled by extending the period of incubation, as the concentration of tin in the liquor will tend to increase during storage.

For the two vegetables reported in this paper, the concentrations of dissolved tin required to inhibit *Cl. botulinum* differ considerably. It is not unlikely, therefore, that a characteristic inhibitory level will be found for each vegetable. Supporting evidence in this regard has already been obtained.

It is also known (Adam and Horner, 1937) that vegetables differ widely in their ability to dissolve tin from tinplate containers. Vegetables which, in plain cans, naturally dissolve tin in concentrations lower than the inhibitory level should regularly permit the growth of *Cl. botulinum*. When vegetables in plain cans naturally dissolve tin in concentrations which approach or exceed the critical levels, growth of *Cl. botulinum* is likely to be irregular or inhibited. The lack of precise information on the rates of dissolution of tin and of germination of spores renders it unwise to assume that *Cl. botulinum* would always fail to grow in vegetables which naturally attain an inhibitory level.

5. Acknowledgments.

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The Use of the Sharples Supercentrifuge in the Fractionation of Colloidal Material.

*By J. S. Hosking, M.Sc., Ph.D.**

Summary.

A method employing the supercentrifuge is described for the fractionation, and determination of the particle size distribution, of colloidal material. The mean particle sizes of the separated fractions determined by ultramicroscopic count agree well with those calculated from the supercentrifuge data. This clearly justifies both the use of the instrument and the simple application of Stokes' Law in the quantitative separation of colloidal particles, and in the determination of their size from the velocities of sedimentation in the centrifugal field.

1. Introduction.

In the absence of direct methods of observation, indirect ones have usually to be adopted for the determination of the size of particles in any polydisperse colloidal system. In general, these latter methods depend upon the application of either Stokes' Law or the ultramicroscopic count formula, for both of which, however, a spherical shape must be assumed, although it is realized that the departure from the spherical may be quite appreciable in most instances, particularly for soils and clay mineral colloids where plate- or rod-shaped particles predominate.

The exact definition of the size of such irregular shaped particles has been the subject of much consideration, and as a result thereof the "equivalent radius" or diameter has been universally adopted as the most convenient and easily appreciable standard, both for the expression of the size of the individual grains or groups of particles in any system, and for the direct comparison of the size of the particles of different materials. The "equivalent radius" is taken as the radius of a sphere of the same material, and having, in the case of determination by sedimentation, the same settling velocity or, in the case of ultramicroscopic observation, the same volume, as the particles concerned.

For the actual separation of clays and soil colloids into various fractions, both the centrifuge and supercentrifuge have been used frequently. In the main the separations have been essentially on a qualitative basis, although methods have been devised whereby the actual particle size of distribution could be obtained with a reasonable degree of accuracy.

During the course of an investigation into the properties of kaolinitic colloids (Hosking, 1942), it was necessary not only to fractionate large quantities of the colloids but also to obtain a definite measure of the size of the particles of each component fraction. It was decided to use the Sharples supercentrifuge rather than the ordinary centrifuge for the purpose, since the former offered the advantages of a much higher angular velocity, and hence higher rate of sedimentation, and possessed the means whereby continuous flow of the colloidal

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suspensions through the instrument could be obtained. The adaptability of the instrument to quantitative separation is illustrated by the following results.

2. Material Examined.

The materials employed in the work were (*a*) the colloid fraction of a sample of ground kaolinite, the original sample of which came from near Langley in South Carolina, and (*b*) the colloid fraction separated from a sample of the Aiken soil obtained from near Morgan Hill in California. The colloid fractions were separated from the coarser materials of the samples by repeated sedimentation of the samples through 8.6 cm. in 24 hours, using small amounts of sodium carbonate as dispersing agent. The samples were dispersed in several litres of distilled water in 5-gallon jars by means of mechanical agitation, and the process of dispersion, sedimentation, and siphoning off of the solutions was continued until the colloids remaining in suspension were reduced to negligible amounts.

Following coagulation of the suspensions of the separated colloids with calcium chloride and removal of carbonates with a minimum amount of dilute hydrochloric acid, the supernatant liquid was drawn off and the colloids were concentrated by vacuum filtration after washing with distilled water to remove the excess calcium chloride. The Aiken colloid was then subjected to treatment with hydrogen peroxide on the steam bath in order to remove organic matter.

Samples of each of the clay pastes were then electro-dialysed until completely free from excess salts and exchangeable ions, and the products for fractionation stored in suspension. The remainder of the samples were calcium-saturated, air-dried, and stored for subsequent examination.

Assuming the applicability of Stokes' Law, the upper limit to the size of these colloid fractions is given by the equation—

$$d = \sqrt{\frac{18\eta v}{(\rho_p - \rho_l)g}} \quad \ldots\ldots\ldots (1)$$

where d = diameter of the particles in cm., v = velocity in cm. per sec., η = viscosity of the suspension in poises, ρ_p = density of the particle, ρ_l = density of the liquid, g = acceleration due to gravity. Here, v has the value given above (8·6 cm. in 24 hrs.), g = 980·0 cm. per sec.^{2} (at Berkeley in California), ρ_p and ρ_l have the values given in Tables 1 and 2, and η = 0·978 centipoises, at a temperature of sedimentation varying within the narrow limits of 21° C. to 24° C. with a mean of 22° C. Hence, the maximum equivalent diameters of the particles are 1·18 μ for the the ground kaolinite and 1·07 μ for the Aiken colloid.

3. Fractionation with the Supercentrifuge.

Alternate methods of separation by the supercentrifuge have been employed from time to time. In one, the material is deposited on a sleeve within the bowl of the instrument, and the fractionation of the colloid depends upon the subdivision of the material at definite distances along the length of the bowl (Bradfield, 1925; Steele and Bradfield, 1934; Hauser and Reed, 1936). Of the methods embracing this technique, that of Hauser and Reed was aimed at obtaining a high degree of precision, but unfortunately the mathematical calculations are involved and the technique of separation laborious.

In a second method the suspension is passed through the bowl and a single division is made between the smaller particles which issue at the top and the coarser material which is deposited within the bowl (Bray, 1934; Brown and Byers, 1932; Grim and Bray, 1936; Whitt and Bauer, 1937). Multiple division in this case depends upon the variation of either one or both of the speeds of the bowl or the rate of flow of the suspension, and repeated passage through the machine is necessary before complete separation may be attained.

A third method, an adaptation for the supercentrifuge of the two-layer procedure developed by Marshall (1930) for the centrifuge, has been investigated by Whiteside and Marshall (1939), but it suffers from the disadvantage that only small batches of material may be handled at one time.

The second or multiple-run method is claimed by some to be more tedious than either of the other methods. While this may be true when only small amounts of the various fractions are required, it is by no means so when fairly large quantities are to be separated. The method has the definite advantage over the first that it enables more reliable separation to be made, and over the third, in addition, that complicated calculations are obviated. The principles upon which the separation by multiple division is based are straight forward, and in so far as practical accuracy is concerned only the simplest relationship between the size of the particle and its sedimentation velocity in a centrifugal field need be considered.

When considering a particle in suspension in a rotating centrifuge, the acceleration of the particle is not constant as it is in a gravitational field, but varies with its distance from the centre of the centrifuge. Hence the velocity of sedimentation can no longer be taken as a constant, but is equal to the differential $\frac{dx}{dt}$, where x = the distance in cm. of the particle from the surface of the liquid towards the periphery of the centrifuge bowl, and t = the time taken for the particle to travel that distance. When the supercentrifuge is in operation, there is a cylinder of space in the centre of the bowl, the diameter of which is equal to that of the circle of openings in the top of the bowl. The relationship between the radius of a spherical particle and its velocity towards the periphery of the bowl during sedimentation is therefore given by the modified Stokes' formula (Svedberg & Nichols, 1923) as follows:—

$$6\pi r\eta \frac{dx}{dt} = \frac{4}{3}\pi r^3(\rho_p - \rho_l)4\pi^2 n^2(x + a) \quad \ldots\ldots\ldots \quad (2)$$

where $4\pi^2n^2(x + a)$ = the acceleration in the centrifugal field, and replaces g in the ordinary Stokes' formula, and n = the number of revolutions of the bowl per second, a = the distance in cm. from the axis of rotation of the bowl to the surface of the liquid, and the remaining symbols have the significance already given.

This on cancellation, transformation, and subsequent integration reduces to—

$$t = \frac{9\eta \ln\left(\frac{x + a}{a}\right)}{8\pi^2 n^2 r^2(\rho_p - \rho_l)} \quad \ldots\ldots\ldots \quad (3)$$

which gives the time taken for a particle of radius r to move out a distance x from the surface of the liquid. If x is equal to the total

depth of liquid in the bowl, t is the time taken for a particle to just settle at the periphery; in other words if such a particle be initially at the surface of the suspension as it enters at the bottom of the bowl and just settles as the liquid emerges at the top, then t is the time taken for a complete refilling of the bowl.

By means of formula (3) it is thereby possible to determine the speed of the centrifuge and the rate of flow of the suspension necessary to make divisions at any specific particle size in any polydisperse system.

For separation of the colloids into four fractions with the following approximate limits to the equivalent diameters of their individual particles: (i) 1μ to $\cdot5\mu$, (ii) $0\cdot5\mu - 0\cdot25\mu$, (iii) $0\cdot25\mu - 0\cdot1\mu$, (iv) less than $0\cdot1\mu$, about 200 gm. (on an air-dried basis) of the electrodialysed material in concentrated suspension was diluted to about 20 litres, to give an approximately 1 per cent. suspension. This diluted suspension was dispersed by mechanical agitation following the addition of a little normal sodium hydroxide and then passed through the supercentrifuge under conditions determined to give separations at the diameters required. A celluloid sleeve fitting snugly inside the bowl permitted the easy removal of the colloid deposited thereon. In order that the bowl should not become too heavily charged, leading to appreciable errors in separation, it was found advisable to dismantle the instrument several times during each of the earlier passages through it of the 20 litres containing the whole colloid sample. As fractionation proceeded, the number of interim dismantlings necessarily decreased, until for the final separations the whole volume of suspension could be comfortably passed through the bowl in one operation, having been gradually reduced from 20 litres to 5 litres to maintain approximately the initial concentration. Following each run the total colloid deposited on the bowl was removed, well rubbed up with a rubber pestle, rediluted, redispersed by mechanical agitation and the addition of a few drops of normal sodium hydroxide, and again made up to volume.

For the separation of each fraction, the suspension was passed through the bowl repeatedly until the issuing suspension contained less than one part in 10,000 of the colloid. As with the parent colloids, the individual fractions after estimation of the total amount of each were coagulated and filtered, and hydrogen- and calcium-saturated samples were prepared of each for subsequent examination.

For each of the colloids a first division was actually sought at a diameter of $50\text{m}\mu$; however, with the calculated rate of flow and supercentrifuge speed the liquid issuing from the bowl was perfectly clear, and inspection of the sleeve showed, in both cases, that all the colloid had been deposited within about 6 cm. of the bottom of the bowl. This fact indicated that the lower extreme particle size for both the kaolinite and the Aiken colloid was equivalent to a diameter of $60\text{m}\mu$,

Data relevant to the separation of the colloids are given in Table 1, while the percentage distribution and the limiting diameters of each fraction, together with the mean particle size of each fraction calculated from these figures, are shown in Table 2. The evaluation of the viscosities of the suspensions and the densities of the colloidal fractions from physical tables at the time of making the separations necessarily introduced slight errors. However, with the eventual exact determination of these properties on the samples obtained, their substitution in equation (3) enabled the calculations of the more correct values to be made.

The slightly lower dimensions of each of the Aiken fractions compared with those of the kaolinite are imposed primarily by the higher densities of the constituent iron and aluminium oxides and hydrated oxides, haematite, goethite, and diaspore, which amount to about 30 per cent. in the Aiken colloid.

TABLE 1.—DATA RELATING TO THE SUPERCENTRIFUGE FRACTIONATION OF THE COLLOID FRACTIONS.

Radius of the supercentrifuge bowl $(x + a) = 2{\cdot}1875$ cm.
Radius of air space in the bowl $(a) = 0{\cdot}755$ cm.
Effective length of the bowl $(l) = 19{\cdot}650$ cm.
Volume of the bowl $(v) = 260{\cdot}2$ c.c.
Density of Water (ρ_l) at 24° C. to 25·5° C. = 0·997 ; at 27·5° C. = 0·996.

Sample.	Division Sought at Diameter (d).	Mean Temperature during Operation (T).	Mean Viscosity of Suspension (η).	Mean Velocity of Centrifuge (n).	Mean Density of Particles (ρ_p)*.	$\rho_p - \rho_l$.	Time for Liquid to Pass through the Bowl (t).	Times Suspension Passed through Bowl.	Mean Size of Actual Division. Diameter (d).
	μ	° C.	Centi-poise.	r.p.s.			Secs.		μ
Ground Kaolinite..	0·10	24·0	0·934	633	2·250	1·253	80·7	6	0·106
	0·25	25·5	0·899	532	2·296	1·300	15·6	15	0·275
	0·50	25·5	0·893	270	2·347	1·350	15·6	20	0·529
Aiken Colloid ..	0·10	24·0	0·934	633	2·530	1·533	80·7	6	0·096
	0·25	24·3	0·928	529	2·554	1·557	15·6	15	0·258
	0·50	27·5	0·845	270	2·561	1·565	15·6	20	0·480

* The density shown is the mean density of the material remaining in suspension after the removal of the smaller sized fractions and is not that of any particular fraction, which is shown in Table 2.

4. Mean Particle Size—Ultramicroscopic Observation.

In order to make a check on the particle sizes as calculated from the supercentrifugal separation, the mean size of each fraction was determined by means of the ultramicroscope.

The ultramicroscopic count was carried out by using a Zeiss slit ultramicroscope. The high power immersion system was used throughout, and each series of determinations comprised from 500 to 1,000 counts. Colloidal solutions of such dilution as to allow ease of counting were prepared from standard suspensions containing the equivalent of 1 g. of sodium-saturated air-dry colloid per 100 c.c., which had themselves been prepared by adding the equivalent amounts of sodium hydroxide to the electrodialysed samples in suspension.

From these counts the actual calculation of the mean particle size depends simply upon equating the weight of the particles, determined from the total number of particles, to the weight calculated from the concentration of the suspension, i.e., if d = the diameter of a spherical particle in cm., ρ_p = the density of the particle, N = mean number of particles present in the selected volume, c = the concentration of the dispersed phase in g. per c.c., and V = the selected volume of the suspension in c.c. in the Tyndall beam, then—

$$d = 2\sqrt[3]{\frac{3cV}{4\pi N\rho_p}} \quad \ldots \ldots \quad (4)$$

The values obtained in this way are shown in Table 2 alongside those determined from the supercentrifuge sedimentation for comparison.

5. Conclusions.

From Table 2 it is apparent that there is reasonable agreement between the values calculated from the distribution curves and those determined by ultramicroscopic count. The figures, however, for the two fine colloid fractions and for the kaolinite coarse colloid fraction call for some comment.

TABLE 2.—PERCENTAGE DISTRIBUTION OF THE COLLOID FRACTIONS AND COMPARISON OF PARTICLE SIZE AS DETERMINED BY THE SUPERCENTRIFUGE AND ULTRAMICROSCOPE.

Material.	Subfraction.	Density of Sub-fraction.	Per-centage Distri-bution.	Particle Size. Equivalent Diameters.		
				Supercentrifuge.		Ultramicroscope.
				Limiting Value.	Calculated Mean Value.	Mean Value.
				μ	μ	μ
Ground Kaolinite	Whole Colloid ..	2·249	..	1·18 to 0·06	0·21	0·24
	Coarse Colloid ..	2·352	15·6	1·18 to 0·53	0·72	0·66
	Medium Colloid..	2·331	15·1	0·53 to 0·28	0·37	0·39
	Medium Fine Colloid	2·208	63·9	0·28 to 0·11	0·19	0·21
	Fine Colloid ..	2·133	5·4	0·11 to 0·06	0·09	0·13
Aiken Soil ..	Whole Colloid ..	2·535	..	1·07 to 0·06	·23	0·23
	Coarse Colloid ..	2·562	15·1	1·07 to 0·48	·63	0·64
	Medium Colloid..	2·559	28·0	0·48 to 0·26	·35	0·34
	Medium Fine Colloid	2·510	40·7	0·26 to 0·10	·18	0·19
	Fine Colloid ..	2·444	16·2	0·10 to 0·06	·085	0·11

It must be realized that the utramicroscopic method is extremely sensitive to the presence of small particles, and in consequence there will always be a tendency for this method to give low values where incomplete separation of smaller sized particles has occurred. On the other hand, high values will be obtained where the particles deviate from the spherical or equidimensional. Generally speaking, the ultramicroscope figures found are the higher, and this is particularly noticeable for the fine colloid fractions. Although in these cases the figures may indicate a much more marked departure from the spherical than for the coarser particles, a fact which was apparent from viscosity considerations (Hosking, 1942), it is considered possible, in the light of the low visibility of these fractions when viewed in the ultramicroscope, that these higher values may be due to the non-registration of a number of the finest particles during counting. With the coarse kaolinite colloid fraction, although the lower ultramicroscope value might be considered as being due to the retention of finer grained particles, it is possible that many of the illuminated particles outside the Tyndall beam were actually included in the count, and that this higher count has resulted in a lower value for the particle size than there should have been. During the examination of the coarse kaolinite fraction, it was shown (Hosking, 1942) that the grinding process had caused a marked agregation of the particle to form secondary particles which were extremely resistant to the ordinary methods of mechanical dispersion. In the ultramicroscopic field the presence of what appeared to be clumps of particles was very noticeable, and in many instances where the particles which went to make up these clumps appeared to be distinguishable they were counted as separate entities, when in reality the whole clump should possibly have been considered as a single unit. This latter explanation would appear to be the more reasonable, since the same criterion of minimum scintillation of the particles was taken into account in the case of the Aiken colloid, in assessing whether or not the particle was in the Tyndall beam; the agreement between the ultramicroscope value and the calculated value for the Aiken coarse colloid is, however, quite good.

Apart from these deviations, the agreement between the two sets of values is very satisfactory, and it is apparent that the Sharples supercentrifuge may be employed for both fractionation and the determination of the size of the separated fractions when due attention of course is paid to the accurate estimation of all the factors, viscosities, densities, &c., involved in the calculations. Moreover, the results are shown to justify very clearly the simple application of Stokes' Law in the separation and determination of the size of colloidal particles from the velocities of sedimentation of the grains in the centrifugal as well as in the gravitational field.

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Estimation of Damage to Potato Foliage by Potato Moth, *Gnorimoschema operculella* (Zell.)

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Summary.

1. The leaf area of about 30 potato plants, including six early and late maturing strains and varieties, was measured periodically during the earlier stages of growth. The last of these measurements gave an estimate of the destruction of leaf tissue by larvae of the potato moth.

2. They also gave information on the mode of attack on the host plant.

3. Coincident with the last leaf measurements and until the tops of the earliest strains were nearly dead, ratings of individual plants were made on a 5-point scale according to the extent of leaf area destroyed by larvae of the potato moth.

4. Moth damage ratings were influenced by the growth habit of the infested variety. Varieties with open foliage were rated higher than dense varieties, because the damage could more easily be seen.

5. Moth damage ratings might also be influenced by growth conditions, having little to do with the number of moth larvae.

6. Significant differences in the reaction of strains and varieties to infestation were associated with differences in the rate at which leaf tissues destroyed were replaced by the growth of axillary shoots. There was no evidence that the moths preferred the leaf tissues of one variety more than those of another.

7. The yield of infested plants was approximately proportional to the amount of leaf area left undamaged by the potato moth. This provides a logical basis for the rating method of estimating moth damage.

8. The uses and limitations of the rating method for estimating moth damage are discussed.

1. Introduction.

Growth studies on potato plants (unpublished) have shown that healthy plants grown under uniform conditions, and not infested by larvae of the potato moth, *Gnorimoschema operculella* (Zell.) yield approximately in proportion to their leaf area. It would be expected, therefore, that any agency reducing the leaf area would lower the yield. The larvae of the potato moth, by destroying part of the foliage of infested plants, may substantially reduce the yield. The form of injury they cause is clearly apparent, and its extent can be estimated and given some form of quantitative expression. The amount of leaf tissue destroyed may therefore be used as an indication of the damage caused by the moth in a potato crop; and it might also be used in experimental plots laid out to test treatments for the control of moth, as a measure of the effectiveness of the treatments.

A quantitative estimate of the leaf tissue destroyed by the potato moth is possible only if loss of tissue in infested crops is mainly due to the moth. There are diseases and pests, e.g. early blight, *Macrosporium solani* E. & M., the potato flea bettle, *Xenidia picticornis* Blackb., and the leaf-eating ladybird, *Epilachna 28-punctata* (Fabr.) which also cause destruction of leaf tissue, but there are many regions where the potato moth is the main cause of this kind of damage.

Injury to the above-ground parts of the potato plant is caused by larvae which hatch from eggs laid on the under-surface of the leaves, in leaf axils, or in the soil at the foot of the plant. The larvae mine into the leaf or tunnel in the petiole or stem (Atherton, 1936; Graf, 1917). Mining continues while the larvae remain small, and during this time they feed on mesophyll tissue in interveinal areas, occasionally eating through some of the smaller vascular bundles. This causes infested leaves to shrivel, and produces the characteristic withered or scorched effect seen in infested plants (Plate 1). As the larvae grow, however, the space between the upper and lower epidermal layers of the leaf becomes too small for them, so they either roll adjacent leaves together, as in the unexpanded growing tips, or begin to tunnel in petioles or stems. This form of injury is quite characteristic and differs from that produced by other chewing larvae which bite off and consume entire portions of the leaves of their host.

In practice, the amount of leaf tissue destroyed by potato moth larvae is not easily measurable. It is possible, however, by direct comparison to judge whether one plant is more severely damaged than another. Further, it should be possible to rate plants by comparison with a series of standards which have known proportions of their foliage destroyed, and to use the ratings as a measure of infestation. Similar ratings have been used to measure infestation by pests of other crops (e.g. Hervey, 1943).

Moth damage ratings of this kind were made in 1942-43 during a field test of sprays for use against the potato moth. The standards taken were arbitrary, but to ensure consistency between moth damage ratings made at different times, photographs were taken of plants falling within the classes represented by the different ratings, and were kept for reference. Examples of the photographs are given in Plate 1. The ratings were 0 and 1 to 5, 0 representing a plant on which damage was negligible and 5 a plant of which all or nearly all the leaf tissue was destroyed. The ratings 1 to 4 represented successively greater destruction of leaf tissue. The arbitrary nature of the standards left some doubt of the reliability of the method and of what it actually measured, so an attempt was made to see if the ratings had a consistent quantitative basis.

During February and March 1943, measurements of leaf area were taken in a small plot of potatoes, which included varieties of various growth habits and maturities. Towards the middle and end of March, infestation by the potato moth occurred to a degree that made further measurements valueless for the original purpose of the experiment. However, the plot provided excellent material for a test of the method of rating for damage by the potato moth, and in the later periods of development it was used for this purpose. The growth studies, for which data were gathered during the earlier stages of the experiment, were complementary to the work on rating for moth damage, and the results will be summarized in a later section of this paper. They are described more fully elsewhere (Bald, 1944).

TABLE 1.—LEAF AREA RATINGS FOR EACH LEAF OF ONE SHOOT OF LATE CARMAN PLANT 2–6, MAIN SHOOT AND AXILLARIES. NODES AND LEAVES NUMBERED FROM BASE. PERIOD OF ESTIMATION, FEBRUARY 26 TO MARCH 24.

The Figures in Brackets Represent the Size of the Remaining Portions of Leaves Damaged by Potato Moth Larvae.

Date of Leaf Area Rating.	Main Shoot. Leaf on Node No.																
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	..
26/2 ..	0	0	1	2	5	6	4	5	5	3	1	0	T				
2/3 ..			1	2	5	6	5	6	7	5	5	1	0	T			
9/3 ..				1	5	6	5	7	7	8	4	6	1	1	0	T	
17/3 ..	K	K	K	K	K	(0)	(0)	(3)	(4)	7	8	8	(3)	(5)	8	(2)	F
24/3 ..	K	K	K	K	K	K	K	(2)	(1)	(6)	(6)	(8)	(3)	(5)	(8)	(4)	F
Maximum leaf area rating	0	0	1	2	5	6	5	7	7	8	8	8	3	5	8	4	F

Date of Leaf Area Rating.	Axillaries arising from Node No.		
	3.	4.	5.
26/2	0 T	T	T
2/3	0 0 T	0 T	0 0 T
9/3	0 T	1 0 0 T	0 1 0 T
17/3	K	(0) (2) 4 5 (1) (1) (T)	0 (2) (1) (T)
24/3	K	(0) (1) (3) (5) (3) (4) (2) (1) (T)	K (1) (0) (1)
Maximum leaf area rating	0 0 T	1 2 4 5 3 4 2 1 T	0 2 1 1

Date of Leaf Area Rating.	Axillaries arising from Node No.		
	8.	9.	10.
2/3	T	0 T	T
9/3	2 2 0 T	3 3 1 0 T	T
17/3	4 4 6 7 5 K K	5 7 8 8 7 7 (3) 0 (T)	T
24/3	(4) (0) (6) (7) (8) K K	4 (6) 8 8 (8) (8) (6) 7 (1) 3 1	(T)
Maximum leaf area rating	4 4 6 7 8 0 T	5 7 8 8 8 8 6 7 1 3 1	T

Date of Leaf Area Rating.	Axillaries arising from Node No.			
	11.	12.	16.	16 (*a*)*
2/3	0 T		T	
9/3	1 0 T	T	T	
17/3	4 3 (3) 5 2 1 (T)	K	3 7 0 T	
24/3	(5) (3) (4) (7) 6 7 3 4 1 (0) T	K	5 8 3 1 2 0 T	1 0 T
Maximum leaf area rating	5 3 4 7 6 7 3 4 1 0 T		5 8 3 1 2 0 T	1 0 T

* A secondary axillary arising from the primary axillary of node 16 on the main stem.

TABLE 2.—SCALE OF LEAF AREAS EQUIVALENT TO LEAF AREA RATINGS 0–12.

Leaf Area Rating.	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Equivalent area (sq. cm.) ..	10	21	27	34	43	55	70	89	114	145	184	234	299

2. Methods.

The varieties of potato used in the experiment included four of the six most commonly grown in Australia, and of one, Carman, there were an early and a late strain. Each variety was represented by a single row of 15 plants widely spaced to avoid competition. The varieties, in order of planting from east to west, were Early Carman, Late Carman, Snowflake, Brownell, and Up-to-Date*.

Estimations of the total leaf area of six plants of each variety were made regularly from emergence (February 12 to 16) until full flowering (March 24 and 25) by matching each leaf of the plants with photographic outlines of standard leaves, the areas of which had previously been determined by planimeter. This was also a rating method and has been fully described elsewhere (Bald, 1943). Where ratings of this sort are referred to, they will be called "leaf area ratings" to distinguish them clearly from those which will be called "moth damage ratings". Serious infestation by the potato moth was evident on March 17 and 18, and a note was made for each leaf, whether it was damaged or destroyed. Similar notes were taken at the last estimation of leaf area on March 24 and 25. Data such as are shown in Table 1 were recorded for each shoot of all plants measured.

An estimate of what the area of leaves damaged by potato moth would have been if no destruction of leaf tissue had occurred was made by assuming for each leaf that the maximum area determined at a previous estimation represented the size of the undamaged leaf. The difference between this and the current estimate was taken as the amount of leaf tissue destroyed. For example, in Table 1, leaf 9 on the main shoot was given its highest leaf area rating, 7, on March 2. It was again rated 7 on March 9; on March 17 some of the tissue had been destroyed by potato moth larvae and it was rated 4, and on March 24 most of the tissue was destroyed and it was rated 1. Its maximum size was taken as 89 sq. cm. (see Table 2) and its size on March 24 was taken as 21 sq. cm. (equivalent of leaf area rating, 1). The leaf tissue destroyed was therefore 89–21 = 68 sq. cm. Obviously in many instances, particularly where the destruction of tissue occurred on immature leaves, this would be an underestimate of the extent of damage, but a careful examination of many data suggests it is not a very serious underestimate during the earlier stages of infestation. Later, the cumulative effects of reduced leaf area may distort the whole course of growth and development, and under such circumstances, the estimate of damage will almost certainly be too low.

The most reasonable and consistent standard by which to judge the amount of destruction on an infested plant is the plant as it would be if the leaves present or formerly present on it were all complete and the tissue normal. Such a standard takes no account of the leaves that were not formed, nor of leaves that failed to expand to their potential maximum area, because the growth rate of the plant had been reduced by the destruction of much of its leaf tissue.

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* Local names of the varieties are given. Descriptions are available elsewhere (Bald, 1941).

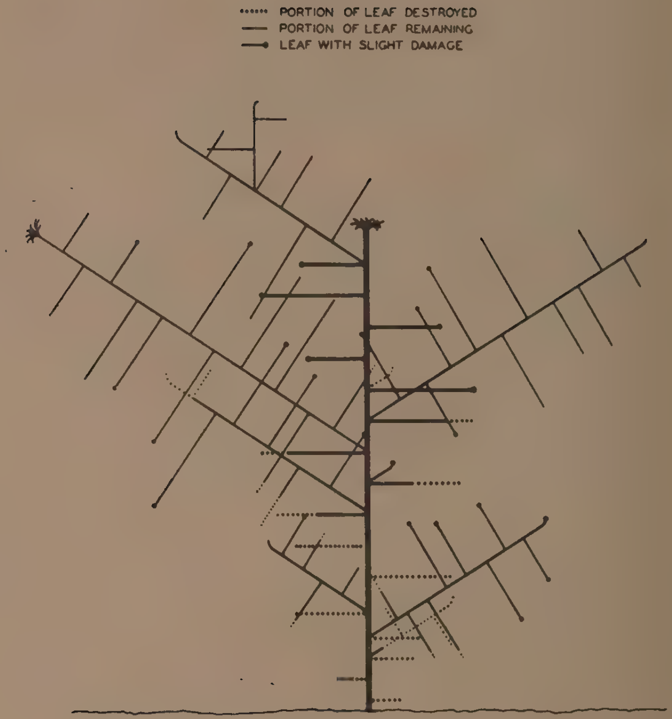

FIG. 1.—Elevation diagram derived from leaf area ratings made on March 24, to show the distribution of moth damage on a single shoot of Late Carman plant 2–6. About 17 per cent. of the leaf tissue has been destroyed. The undamaged leaf tissue is shown by solid lines and the tissue destroyed by dotted lines. The size of the leaves is shown by the length of the lines drawn to represent them; the length has been made proportional to the square root of their area. The phyllotaxy is suggested by drawing successive leaves on the right or left side of the stem as they would appear if the shoot were viewed horizontally from a fixed angle. The gradation of leaf sizes on this shoot is slightly less regular than is usual on a normal vigorous plant, but the shoot illustrates very well the types of damage caused by larvae of the potato moth during the earlier stages of a severe infestation.

This standard was the basis on which the method of rating for moth damage was meant to be founded. The scale, 0 to 5, covered the whole possible range of damage, and the intervals were meant to be equal. If they were really equal in the arithmetic sense, 0 and 5 would represent no damage and complete destruction, 1, destruction of 1–25 per cent. of the leaf area, 2, 26–50 per cent., 3, 51–75 per cent., and 4, 76–100 per cent.

It became possible to test the boundaries of the classes into which plants were rated for moth damage, when ratings were made on the same days as estimations of leaf area (March 24 and 25). Thereafter, although there were no more estimations of leaf area, ratings for moth damage were made on April 3, 9, 19, and 26. These provided data for a study of the damage caused by the potato moth on different varieties (see p. 41).

3. Mode of Attack by the Potato Moth.

The detailed examination of infested plants revealed several important facts about the beginning and development of infestation. These may be illustrated by reference to Table 1, and to the same data in diagrammatic form (Fig. 1). The values given are leaf area ratings made between February 26 and March 24, for all leaves on a single shoot of one Carman plant. The leaf area ratings for leaves recorded as damaged are given in brackets, and K represents a leaf entirely killed. T represents the whorl of rudimentary leaves around a growing tip.

It is evident from Fig. 1 that the moth larvae work up the centre of the plant from the base, and that larvae may also work down from the whorl of leaves around a growing tip. For an individual axillary shoot this is well illustrated by the leaf area ratings of March 17 for the shoot arising from node No. 4 (Table 1). The leaf area rating of March 24 shows (Table 1, Fig. 1) that the moth larvae had not then killed the growing tip, and new leaves had expanded, although every leaf on the shoot had been attacked. On the other hand, the growing tip of the axillary shoot arising from node 8 was killed by March 17, before the leaves towards the base of the shoot were attacked, but by March 24 all leaves on the shoot had been at least slightly damaged.

The axillary shoot from node 12 provides an example of another type of damage where the shoot is killed back almost as soon as it emerges. Sometimes it is killed before any leaves have had time to expand, and therefore it might not even be recorded.

These forms of damage are possibly related to the egg-laying habits of the female moths and the feeding habits of the larvae. Eggs may be laid almost anywhere on the plant (Attia and Mattar, 1939; Graf, 1917), but common sites for egg-laying are the soil at the base of the plant (Atherton, 1936), between the junctions of the leaf axils and the stems, and in the whorls of leaves at a growing tip (unpublished data). Egg laying between the soil and the stem of the plant is likely to occur when the plant is small and provides relatively little shelter above soil level. Larvae emerging from eggs in this position probably invade the lower leaves. Those from eggs laid at the insertion of a leaf axil would naturally invade that leaf or the axillary shoot arising from that node. If the axillary shoot were small, the larvae might invade the tip and kill the growing point; if it were large and vigorous, they would probably start feeding on the lower leaves of the axillaries. Larvae from eggs originally laid in the whorl of immature leaves at the tip of a vigorously growing shoot would not be at the tip when they emerged. The shoot would have grown beyond them, and they would

emerge at the level of the lower or middle leaves. This may explain the presence of many of the newly emerged larvae found on the younger of the fully expanded leaves.

The larvae responsible for the killing back of large rapidly-growing shoots, e.g. that arising from node 8 in Fig. 1, are generally well grown, and may be migrants from other feeding grounds on the plant.

The sites at which the potato moth larvae attack their host plants, and the resultant forms of damage, will be shown later to influence the moth damage rating method and the final estimate of the effects on the host plant of the destruction of leaf tissue. The following aspects of infestation are particularly important. The form of attack in the initial stages of infestation, especially if the plant is growing rapidly, is such that much of the damage done to foliage may be hidden, because it is largely confined to the base and centre of the plant (Fig. 1). New growth, which is less likely to be infested, is largely on the outer perimeter of the plant. The killing of growing tips at various stages may prevent or retard the development of new leaf tissue, or it may merely delay the development of new tissues, and infestation may be obviously severe for a time, but later there will be an apparent recovery. These points will be brought out in detail in later sections of this paper.

4. Rating for Moth Damage.

The first moth damage rating of the leaf area plot was on March 24, when the last estimations of leaf area were made. On March 25, a second moth damage rating was made by the same operator (G.H.) without reference to the first. The results are shown in Table 3. There is no doubt of the consistency with which the moth damage ratings were made. In several instances, where plants were rated differently on the two occasions, they were close to the borderline between two classes (Table 6, 7).

Table 3.—Comparison of Successive Moth Damage Ratings for Individual Plants Made on March 24 and 25.

Variety.	Row.*	Number of Plant in Row.														
		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
Early Carman	1	2, 2	..	2, 2	2, 2	..	2, 2	3, 3	2, 2	1, 2	3, 3	2, 2	2, 2	2, 2	2, 2	3, 3
Late Carman	2	4, 4	2, 2	2, 2	1, 1	..	1, 1	1, 2	2, 2	2, 2	2, 2	2, 2	3, 3	1, 1	1, 2	2, 2
Snowflake	3	3, 3	..	2, 2	2, 2	2, 2	2, 2	2, 2	2, 2	2, 2	..	2, 2	1, 1	..	1, 2	1, 1
Brownell	4	3, 3	3, 3	2, 2	2, 2	2, 2	1, 2	2, 2	2, 2	2, 2	2, 2	..	2, 2	2, 2	2, 2	2, 2
Up-to-Date	5	..	2, 2	..	2, 3	2, 2	2, 2	2, 2	2, 2	..	..	..	2, 2	..	..	..

* Row 1 was on the east side of the plot and plant 1 on the northern end of the row.

The consistency of moth damage ratings made at greater intervals of time is not so easily tested, but there is evidence that during the period of this experiment there was little variation of standards. During two periods, April 3 to 9 and April 19 to 26, there was only slight increase in damage and the ratings given are shown in Tables 4 and 5. Their consistency is obvious.

TABLE 4.—COMPARISON OF SUCCESSIVE MOTH DAMAGE RATINGS FOR INDIVIDUAL PLANTS MADE ON APRIL 3 AND 9.

Variety.	Row.	Number of Plant in Row.														
		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
Early Carman	1	3, 4	..	4, 5	4, 5	..	3, 3	5, 5	3, 3	2, 2	4, 5	3, 3	2, 3	2, 3	3, 3	4, 5
Late Carman	2	5, 5	3, 3	3, 3	3, 3	..	2, 2	2, 3	2, 2	2, 3	2, 3	3, 4	4, 3	2, 2	2, 2	2, 2
Snowflake	3	4, 4	..	3, 3	2, 3	2, 3	2, 3	2, 2	2, 2	2, 2	..	2, 2	2, 2	..	2, 2	2, 2
Brownell	4	3, 3	3, 3	3, 3	2, 3	3, 3	2, 2	3, 3	3, 3	3, 2	2, 2	3, 3	..	3, 3	3, 3	3, 3
Up-to-Date	5	..	3, 3	..	4, 4	4, 4	3, 3	3, 3	3, 3	..	..	..	2, 3	..	..	3, 3

TABLE 5.—COMPARISON OF SUCCESSIVE MOTH DAMAGE RATINGS FOR INDIVIDUAL PLANTS MADE ON APRIL 19 AND 26.

Variety.	Row.	Number of Plant in Row.														
		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
Early Carman	1	5, K	..	5, K	5, K	..	4, 5	5, K	4, 5	3, 4	5, K	4, 5	5, K	4, 5	4, 5	5, K
Late Carman	2	5, K	4, 4	4, 4	3, 3	..	3, 3	4, 4	3, 3	3, 3	3, 4	4, K	4, 5	3, 3	3, 3	3, 4
Snowflake	3	4, 4	..	3, 4	3, 3	3, –	3, 3	3, 3	3, 3	3, 3	..	3, 4	3, 3	..	3, 3	3, 3
Brownell	4	4, 4	4, 4	4, 4	4, 4	3, 3	3, 3	4, 4	4, 4	3, 3	4, 4	4, 4	..	3, 3	3, 3	4, 4
Up-to-Date	5	..	3, 3	..	5, K	4, 4	3, 3	3, 3	3, 4	..	..	..	3, 4	..	..	3, 3

K indicates the death of all tissues above ground level.

5. Estimation of Leaf Area Destroyed by Conversion of Moth Damage Ratings.

Figures for the destruction of leaf tissue by the larvae of the potato moth were obtained from estimations of leaf area on March 24 and 25, and were compared with the successive ratings for moth damage made on the same days. The results are shown in Table 6. Originally, six plants of each variety were measured, but two Early Carmans infected with a virus disease, rugose mosaic, were also measured. One plant amongst the Snowflakes chosen was found to belong to another variety and was discarded. Thus in Table 6, there are values for eight Early Carman plants, and five Snowflakes; and there are values for six plants of the other varieties.

Examination of the data in Table 6 reveals that higher moth damage ratings were given to equally damaged plants of Snowflake and Brownell than of the other four varieties. This was particularly true of the Snowflake plants. The reason may be seen by reference to Fig. 2 and a description of the growth habits of the different varieties. Carman and Up-to-Date form upright and compact bushes through which damage to the lower leaves and central portion of the plant is not easily visible. The habit of Brownell plants is upright and open, and such damage is more readily seen. The habit of Snowflake plants is open and at first upright, but towards maturity the stems become

semi-prostrate; the main stems normally fall outward exposing the basal leaves and central shoots, and damage is obvious. Thus, the same moth damage ratings for three types of varieties, the upright and compact, the upright and open, and the semi-prostrate and open, represent different degrees of damage.

Three different scales for moth damage ratings of the three types were prepared, largely from the data in Table 6. They are shown in Table 7. The scale for the upright and compact type of plant is very similar to the original division into classes by the limits 0, ¼, ½, ¾, and total destruction (p. 34). Estimates of the percentage of leaf tissue destroyed, derived from these scales, agree fairly well with estimates made directly from ratings for leaf area. (Table 8). To illustrate this, estimates of percentage of leaf tissue destroyed were obtained: (i) from the original leaf area ratings as described on p. 33; these are given for individual plants in Table 6; (ii) from a direct conversion of the moth damage ratings shown in Table 6, by substituting the appropriate mean percentages from Table 7; (iii) by first assuming the estimate (i) for each plant was correct, and then correcting the moth damage ratings in Table 6, where necessary according to the scales in Table 7. The appropriate mean percentages from Table 7 were then substituted for the corrected moth damage ratings. For example, Late Carman 2–4 had been rated 1 for moth damage and in estimating (ii) above, a value of 15 per cent. was given for this plant. But the estimate of damage derived from the leaf area ratings was 30 per cent. (Table 6). According to the first scale in Table 7, the plant 2–4 fell within the class rated 2. For the estimate (iii) above, the value 2 was assumed to be the correct moth damage rating, and the corresponding value, 38 per cent. of the leaf tissue destroyed, was written down for this plant, instead of 15 per cent. The third series of values illustrates the error due to grouping the data into a few classes with wide limits, as distinct from errors due to incorrect moth damage rating. Varietal averages calculated from the data in Table 6 by each of the three methods mentioned above are shown in Table 8. They agree within reasonable limits.

TABLE 6.—COMPARISON BETWEEN MOTH DAMAGE RATINGS AND PERCENTAGES OF LEAF TISSUE DESTROYED OBTAINED FROM LEAF AREA RATINGS. INDIVIDUAL PLANTS OF FIVE STRAINS OR VARIETIES OF POTATO, MARCH 24–25, 1943.

Early Carman.			Late Carman.			Snowflake.			Brownell.			Up-to-Date.		
No.	Percentage Leaf Tissue Destroyed.	Moth Damage Rating.	No.	Percentage Leaf Tissue Destroyed.	Moth Damage Rating.	No.	Percentage Leaf Tissue Destroyed.	Moth Damage Rating.	No.	Percentage Leaf Tissue Destroyed.	Moth Damage Rating.	No.	Percentage Leaf Tissue Destroyed.	Moth Damage Rating.
1– 3	48	2, 2	2– 2	25	2, 2	3– 1	58	3, 3	4– 1	35	3, 3	5– 2	49	2, 2
1– 6	33	2, 2	2– 3	40	2, 2	3– 3	26	2, 2	4– 2	46	3, 3	5– 4	57	3, 2
1– 8	37	2, 2	2– 4	30	1, 1	3– 4	18	2, 2	4– 3	28	2, 2	5– 5	32	2, 2
1–13	24	2, 2	2– 6	15	1, 1	3– 9	21	2, 2	4– 9	35	2, 2	5– 6	25	2, 2
1–14	27	2, 2	2– 9	34	2, 2	3–14	14	2, 1	4–13	26	2, 2	5– 8	53	2, 2
1–15	43	3, 3	2–14	23	2, 1	..	..	..	4–14	30	2, 2	5–12	23	2, 2
7*	66	3, 3	..	..	..	..	..	..	..	..	..	..	..	..
10*	58	3, 3	..	..	..	..	..	..	..	..	..	..	..	..

* Infected with rugose mosaic.

TABLE 7.—SCALES FOR THE CONVERSION OF RATINGS FOR MOTH DAMAGE TO PERCENTAGE OF LEAF TISSUE DESTROYED.

Different Scales are given for Varieties with Different Growth Habits.

Moth Damage Rating.	Growth Habit.					
	Upright Compact.		Upright Open.		Semi-Prostrate Open.	
	Limits Moth Damage.	Mean Moth Damage.	Limits Moth Damage.	Mean Moth Damage.	Limits Moth Damage.	Mean Moth Damage.
	%	%	%	%	%	%
0	0– 5	2	0– 5	2	0– 5	2
1	6– 25	15	6– 20	12	6– 15	10
2	26– 50	38	21– 40	30	16– 35	25
3	51– 75	62	41– 70	55	36– 65	50
4	76– 95	85	71– 95	82	66– 95	80
5	96–100	98	96–100	98	96–100	98

TABLE 8.—COMPARISON BETWEEN MEAN VALUES FOR PERCENTAGE DESTRUCTION OF LEAF TISSUE FOR FIVE STRAINS OR VARIETIES OBTAINED (i) DIRECTLY FROM LEAF AREA RATINGS, (ii) FROM CONVERSION OF MOTH DAMAGE RATINGS, (iii) FROM CONVERSION OF CORRECTED MOTH DAMAGE RATINGS (SEE TEXT). DATA FROM TABLES 6 AND 7.

Variety.	Percentage of Leaf Tissue Destroyed by Potato Moth Larvae.		
	Estimate from Leaf Area Rating.	Estimate from Moth Damage Rating.	Estimate from Corrected Moth Damage Rating.
	%	%	%
Early Carman	42·0	47·0	41·1
Late Carman	26·5	28·2	28·3
Snowflake	27·0	28·0	27·4
Brownell	34·2	38·3	33·3
Up-to-Date	38·3	40·0	40·0
Mean	33·6	36·3	34·0

6. Progressive Destruction of Potato Foliage.

With the aid of the values given in Table 7, the moth damage ratings made on the different varieties of potato were converted to percentages of leaf tissue destroyed. The results obtained from an analysis of the percentages illustrate difficulties that must be considered in establishing a rating method for the estimation of moth damage.

Examining the block of plants as a whole, there appeared to be an edge effect at the northern end of rows, where one or two plants in each row became very heavily infested (Tables 3–5). The edge effect was not nearly so evident at the southern end of the block. Discarding two end plants on the northern and one on the southern end of each row, the remaining twelve positions were divided into lots of four, making up a northern, a central, and a southern block of plants. Within each row, i.e. each variety, the lots of four plants served as replicates. A mean value for percentage of leaf tissue destroyed at each date was

obtained for all plots. In one instance (the Up-to-Date plot at the south end of the row) only one of four plants grew, and by itself it provided the value for percentage of leaf tissue destroyed. However, it can be seen from Tables 3–5 that the majority of values for the five varieties were derived from three or four plants. To make sure that bulking did not significantly affect the means or estimates of error, trial analyses were also made, using values for single plants. The results of the two methods agreed very well.

The mean values in Fig. 2 illustrate the progress of infestation throughout the plot as a whole. Serious destruction of leaf tissue began between March 9 and 17. It proceeded steadily until April 3, then slackened for a few days. After April 9, it began again at a somewhat lower rate, which was further reduced between April 9 and 26. From then until the plants were killed by frost, there was very little further destruction of leaf tissue by potato moth larvae.

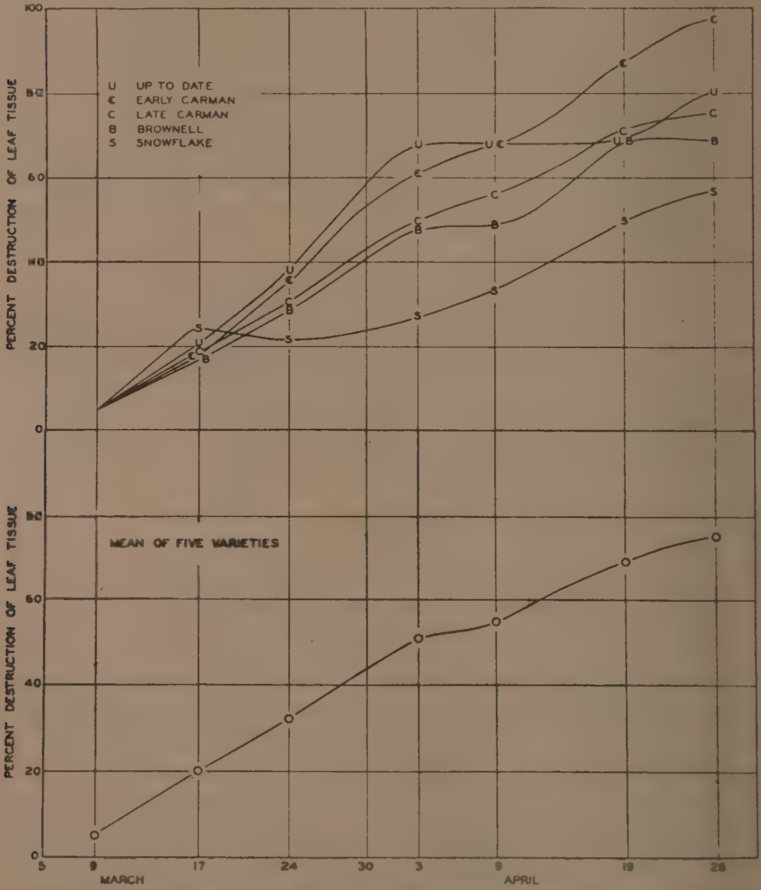

FIG. 2.—Graphs showing the progressive destruction of leaf tissue by larvae of the potato moth. Above is a comparison of five different strains and varieties, below the mean values for all varieties taken together. For details see text.

The form of this curve may have been partly determined by temperature, but the development of the potato moth must also be taken into account. From February 16, when the majority of plants were well above ground, until April 4 when the first pause in the destruction of leaf tissue began, was a period of 48 days. The mean temperature for the period was 22°C. According to Attia and Mattar (1939), at this temperature the full life cycle of *Gnorimoschema operculella* should occupy about 45 days. Therefore, it is probable that many of the larvae feeding between April 9 and 19 were the second generation to develop on these plants, and it is possible that the eggs from which they hatched were laid after differences had developed between varieties in the size of plants and the amount of leaf tissue destroyed. It cannot be assumed, however, that the period April 4 to 9, when relatively little leaf tissue was destroyed, represented merely a pause between generations, as it included a period of three days, April 6 to 8, when the mean temperatures were 10°C. or lower. The maximum temperatures on two days were below the critical temperature for development, 15°C. (Attia and Mattar, 1939). Temperatures below this level also occurred for five of the seven days between April 19 and 24, when again the rate of destruction was low. Such temperatures slow down, but do not interrupt, the regular development of the potato plant; hence their occurrence during periods when infested plants are still capable of vigorous growth might reduce the appearance of damage without reducing the infestation. Moth damage ratings before and after such a period would not alone distinguish between apparent recovery and recovery following an actual decrease of infestation.

Although the rate of destruction of leaf tissue may be subject to fluctuations produced by responses of the potato moth larvae to conditions of temperature, there may be irregularities of another kind in which the insects themselves are not directly concerned. Differences in growth rate arising from variations in soil moisture or soil fertility will cause variations in the rate of replacement of damaged leaf tissue by the growth of new leaves. Differences, probably of this kind, were found between the northern and central blocks of the experiment. They were evident between March 25 and April 9, and were traced to uneven irrigation on March 12, and irregular drying out in the subsequent dry period. They disappeared in the latter part of April after sufficient rain had fallen.

The conclusion that must be drawn from these examples is that the destruction of leaf tissue as estimated by the moth damage rating method cannot, without critical examination, be used as a direct measure of infestation.

7. Varietal Reactions to Infestation.

Comparison between the reactions of the different varieties in this experiment is possible for the period between the dates March 17 and April 26. In making them it must be remembered that varietal differences are confounded with position because of the arrangement of the varieties in rows. Therefore, weight has been placed more on a comparison between the destruction of foliage by infestation and the growth habits of the varieties than on the usual criteria for the significance of differences.

Estimates of moth damage for March 17 and 24–25 were obtainable directly from the estimation of leaf areas, and moth damage ratings supplied data from March 24–25 to April 26 inclusive (Fig. 2). On March 17, there was no evidence of varietal differences; for all varieties the amount of destruction was near to 19 per cent. On March 24–25 differences began to appear, and these remained consistent over the period ending April 9. The varieties fell into three groups: Early Carman and Up-to-Date were most severely damaged, Late Carman and Brownell less damaged, and Snowflake least of all. In the third period, April 10–26, the relative position of Up-to-Date changed, the rate of destruction fell until this variety was not much more seriously damaged than Late Carman and Brownell. Still, Early Carman was more severely damaged and Snowflake less severely damaged.

The internal variability of the experiment leaves little doubt that most of these differences were real, but does not decide whether they were due entirely to varietal differences. However, there were significant associations between the amount of destruction and features of the plants' development, having nothing to do with the severity of infestation, that suggested the differences were dependent on varietal characteristics. Some of these data are shown in Table 9.

In the last column is the rate of increase in leaf area of axillary shoots from about February 27 to March 24 or 25. In column 2 is the mean number of leaves, arising from nodes at the base of the main axes of measured plants, that were completely destroyed, and in column three is the mean number of basal axillary shoots that failed to develop, or had been killed back by March 24 or 25. In the other three columns are percentage destruction of foliage on March 24–25, April 3–9, and April 19–26.

The numbers of basal leaves destroyed are about the same for all varieties, but the numbers of axillaries lost are significantly different. There is an obvious association between the numbers of basal axillaries lost* and the total destruction of foliage in the earlier stages, and an association between the axillary growth rate and the final figures for the destruction of leaf tissue. For the varieties other than Up-to-Date, there is an association between all the varieties in Table 9 except the number of basal leaves destroyed. The results of growth studies reported elsewhere (Bald, 1944) adequately explain these facts.

In the development of the potato plant, the increase in the leaf area of the main axes of different varieties, and of early and late strains of the same variety begin at a similar rate. The number of leaves on the main axes is about the same for all varieties, and on varieties having leaves of the same average size the leaves go through a similar cycle of growth. Differences appear only in the rate of axillary growth. The time at which rapid growth of axillary shoots begins is the same for early and late maturing strains, provided again that they possess leaves of the same average size on the main axes. In a variety that

* In testing this association and differences between varieties in number of basal axillaries lost, log values were found to give a linear regression and equalize variances.

develops very large leaves (Up-to-Date), the inception of rapid axillary growth is delayed by the continued expansion of the leaves on the main shoot, but when rapid axillary growth does begin it proceeds at a rate corresponding with the maturity of the variety. If the variety is early maturing, the rate is relatively slow; if it is late maturing it is relatively fast.

TABLE 9.—COMPARISON BETWEEN DESTRUCTION OF LEAF TISSUE OF FIVE POTATO VARIETIES BY LARVAE OF THE POTATO MOTH, AND THE LEAF AREA GROWTH RATE FOR AXILLARY SHOOTS. VALUES FOR LEAF TISSUE DESTROYED AT SUCCESSIVE DATES IN APRIL WERE AVERAGED.

Variety.	*Mean No. Leaves on Main Axis Destroyed 24/3.	Mean No. Basal Axillaries Destroyed 24/3.	Percentage Total Leaf Area Destroyed.			Axillary Growth Rate 27/2–24/3.
			24/3–25/3.	3/4 and 9/4.	19/4 and 26/4.	
Early Carman ..	5·1	2·8	36	63	91	·037
Late Carman ..	5·5	1·9	31	53	73	·053
Snowflake ..	5·1	0·9	23	30	53	·061
Brownell ..	6·1	1·3	29	49	69	·052
Up-to-Date ..	5·7	4·2	39	67	75	·052

* Exclusive of leaves destroyed as a result of apical infestation.

Of the varieties in this experiment, Early Carman is an early strain, and Late Carman a late strain of the same variety; Brownell and Up-to-Date are late varieties; and Snowflake, very late. At the last two ratings this is the order in which they are placed by their reactions to infestation. Varietal differences are, therefore, probably not due to preferences of the insect for particular varieties, but to the varieties' ability, by growth of axillary shoots, to replace the tissue destroyed.

The reasons for the anomalous reaction of the variety Up-to-Date during the earlier stages of infestation can now be explained. Serious infestation first occurred at a time when rapid axillary growth was beginning. Axillary growth being slower in early varieties, more of the basal axillaries, which are the first to be infested (p. 36), were destroyed in the early variety than in the later ones (Table 9, Column 3). The delay in the inception of axillary growth in Up-to-Date, because of its large-leaved habit, put it, for the time, in the same class as the earliest variety, and many of its basal axillary shoots were killed at an early stage of development.

In Up-to-Date, the effect of this destruction was like the early pruning of axillaries practised in such crops as tomatoes: the inception of rapid axillary growth was delayed, but the plants' reserves were not greatly reduced by the loss of the first axillaries. Later there was a surge of growth that partly compensated for the loss. The proportion of leaf tissue destroyed by the moth larvae was not finally much greater than for the other varieties of similar maturity.

If there is any doubt whether the scales given in Table 7 are applicable to varietal trials under other conditions, there should be no difficulty in discovering whether moth damage ratings on different varieties are comparable. If necessary, a check could be applied by using the leaf area rating as in the experiment described in this paper, or by using some other measure of damage, which would be less laborious than serial leaf-area ratings. Two indications of damage are the number of leaves on the main stalk killed and the number of basal axillaries killed (Table 9). One is a measure depending on a relatively constant feature of the potato plant, the number of leaves on the main stalk, the other is related to the rate of axillary growth and maturity. Values for these forms of destruction on different varieties could readily be obtained by a sampling technique and compared with ratings for moth damage made on the same plants, or with the mean ratings for the varieties. If there were two varieties of similar leaf size and maturity on which a similar number of basal leaves and axillaries were destroyed, and yet one were rated higher for moth damage than the other, it would have to be assumed that estimations of damage to the two varieties were made according to different rating scales. Corrections derived from the counts of main leaves and axillaries destroyed could then be applied to the scales for conversion of the moth damage ratings.

Instead of using the number of basal leaves and axillaries destroyed as a check on these ratings, they could be used under suitable conditions as the primary data for judging differences in varietal susceptibility to moth attack. Ancillary data on leaf size and maturity might be needed, as a check on conclusions made from these measurements.

8. Relation Between Leaf Area and Yield of Infested Plants.

It was suggested in the introductory section of this paper that the reason for adopting the destruction of leaf tissue by larvae of the potato moth as a criterion of infestation was that loss of leaf tissue resulted in a loss of yield. There is ample evidence (unpublished data) to show that healthy potato plants grown under uniform cultural conditions, and not infested by the potato moth, yield approximately in proportion to their leaf area. It remains to be shown that the yield of infested potatoes is related to the area of leaf surface left on the plant by the insects.

For this purpose, data for all the Carman and Brownell plants, of which the leaf area had been measured by the leaf area rating method, were assembled. There were six of the early strain of Carman and six of the late; and of the Brownells, three were early and three late. Graphs were drawn for the leaf area of each plant during the period when infestation was estimated. This period began on March 9 at approximately the same time as the initiation of tuber development. The curves were of the type shown in Fig. 3. A value was obtained for the average leaf area of each plant during a period of 35 days from March 7 onwards, by reading from the graph the leaf area at the middle of each of seven successive five-day periods, and averaging the values so obtained. The mean value for leaf area was then compared with the total yield of tubers from that plant. The log of the mean was

used for the comparison, because, if a direct proportionality exists between leaf area and yield, values for log yield plotted against log leaf area will fall about a straight line of slope one. Fig. 4 shows such a relation exists in spite of much variability in the yields.

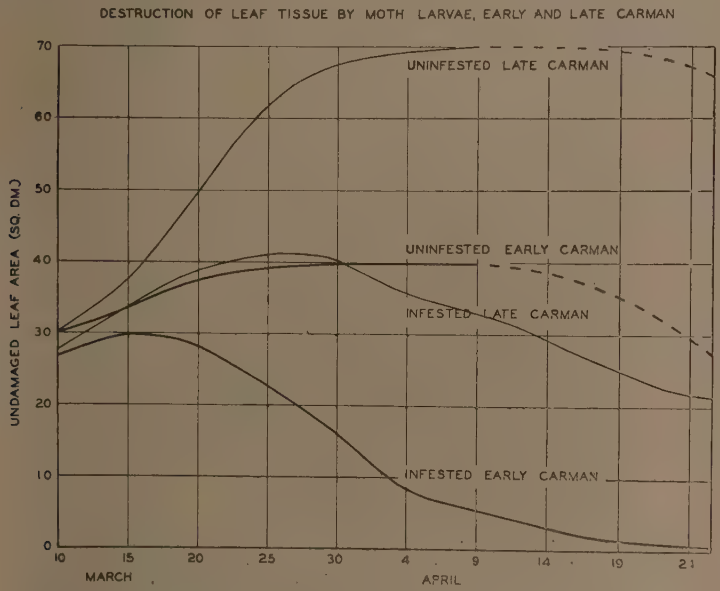

FIG. 3.—Comparison of leaf tissue destroyed by potato moth larvae on Early and Late Carman potato plants. The upper curve for each strain represents the estimated leaf area of a plant if it had not been infested, and, the lower curve, the actual area of leaf tissue remaining on the infested plant. The curves are derived from leaf area and moth damage ratings made on two plants of each strain. Each plant consisted of two main shoots and axillaries. The areas of the main shoots were similar; the difference between varieties depended on the differential growth of axillary shoots.

The extent of the reduction in leaf area caused by the feeding of potato moth larvae is well illustrated in Fig. 3. Fig. 3 is constructed from the mean values for losses of leaf area by two plants of Late Carman (2–4 and 2–9) and two of Early Carman (1–6 and 1–15). Curves drawn for other plants or sets of plants measured had essentially the same features. These pairs of plants were shown in another paper (Bald, 1944) on the average to have emerged on the same day, to have been the same average size during the earliest stages of growth, to have begun rapid growth of axillary shoots on the same day, and to have deviated in size almost solely because of a different axillary growth rate. Data for moth damage showed also that during the earlier stages of infestation the degree of infestation was similar. This is reasonable, because the generation of larvae beginning to feed early in March probably hatched from eggs laid when the Early and Late Carman plants were of the same mean size. On April 4, the same amount of tissue on the

Early Carmans had been destroyed as on the Late Carmans (28 and 29 sq. dm.), but the early Carmans, having a lower rate of replacement, were left with much less leaf tissue (8 sq. dm.) than the Late Carmans (40 sq. dm.). Even the Late Carman plants, however, were so reduced in size that their yield must have been only two-thirds or less of their potential yield.

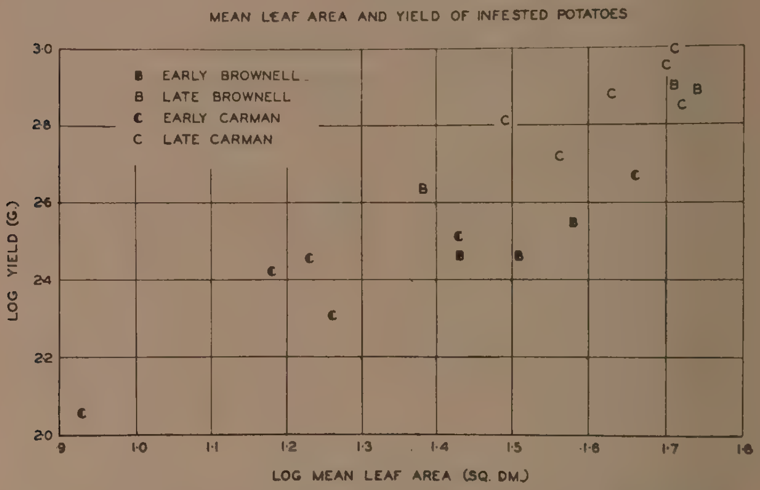

Fig. 4.—Relation between the leaf area and yield of single plants of early and late strains of Brownell and Carman potatoes. The area is given as the log of the mean leaf area during a period of 35 days from the initiation of tuber development.

Summarizing, these data show that a severe infestation of potato moth, on the above ground parts of the potato plant, will cause a large reduction in yield by reducing the leaf area of the plants, quite apart from the damage the moth larvae may cause to the tubers in the ground. Ratings for damage to the foliage will give some indication of the reduction in yield due to attacks by potato moth larvae. Therefore, if proper precautions can be observed in the use of the moth damage rating method, and in the interpretation of the ratings, it will be a useful means of estimating the damage caused by the potato moth.

9. Discussion.

The data given in this paper are derived from the one field experiment involving in all about 60 plants. About half of these were subjected to detailed study. Therefore, the conclusions reached about the relations between growth and infestation cannot be regarded as generally applicable or finally proved. It was not the object of the experiment to reach conclusions of that sort, but rather to discover under local conditions what variates were really measured by the moth damage rating method, whether these variates were closely linked with infestation and the reduction in yield caused by infestation, and to obtain a preliminary definition of the possibilities and limitations of the method of rating for moth damage.

The data show that the method shares a disadvantage common to most indirect methods of measurement, it is subject to error from any cause that affects the chain of association between what is actually measured or estimated and the quantities finally calculated from the measurements. However, there can be no doubt about the association between the leaf area and yield of potato plants, which is the main link in the chain. Whether or not in any particular instance the method of rating for moth damage is used, and what checks are made on the quantities measured, will depend on the object of the studies in progress, and the accuracy required. By means of supplementary estimates of leaf area on single plants and whole plots (Bald, 1943) ratings for moth damage can be made very accurate, and a wide variety of information can be obtained. Types of field experiment in which the method of rating for moth damage is likely to find a place include spraying and dusting trials, and varietal trials. The question of varietal trials has been discussed on p. 44.

In spraying and dusting trials, it is possible under uniform conditions to use the moth damage rating method without even specifying the limits of classes into which the rating divides the plants. Proper replication and randomization of plots would be needed to overcome gradients of soil fertility, as well as unequal concentrations of insects; and to reduce the variability of the experimental material to the lowest possible level a healthy stock of one variety uniform in maturity should be chosen. The question of maturity has been discussed in the body of the paper, but not the question of health.

Data in Tables 3 to 6 suggest there is an association between infection with rugose mosaic in Early Carman and the severity of the damage caused by infestation with the potato moth. Diseased plants are more severely damaged by infestation than healthy. This is likely to be generally true of all diseases that depress the growth rate, by depressing the growth of axillary shoots. Depression of the growth of axillaries is typical of the mosaic diseases and leaf roll, and serious infection with these diseases in stocks used for spray trials is especially to be avoided.

If the experiment is well designed, and the conditions uniform, only an interaction between treatments and the growth of treated plants is likely to upset the rating method for estimating moth damage. There is evidence that one type of spray, that in which copper is an active constituent, prolongs the maturity of the potato plant by increasing the extent of axillary growth. Rating for moth damage, without any check on the meaning of the results, may in this instance, lead to a false interpretation of differences in the proportion of leaf area destroyed. With many other materials there appears to be no such risk. Where there is an interaction between treatment and growth, it is generally quite evident, and allowance can be made for it.

If absolute values for the proportion of leaf area destroyed are needed, either standard photographs (Plate 1) may be used to establish the scale for moth damage ratings, or new standards may be prepared for a particular environment by making serial leaf area ratings on infested plants (p. 33). Such measurements can often be made and standards defined before an experiment is begun. Alternatively, serial

measurements on a sample of plants in the experimental plot could be begun when moth damage was slight and thereafter could be made on the same days as ratings. This would supply a continual check on the consistency and accuracy of the ratings.

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Rubber from Plant Sources.

Investigations on Cryptostegia, Kok-saghyz, and Guayule, with a Note on Synthetic Rubber Research.

1. Cryptostegia.

In April, 1942, a survey was initiated in co-operation with the Queensland Department of Agriculture and Stock with a view to determining the possibility of producing rubber from indigenous trees and shrubs in Australia. The survey disclosed that while there were a number of indigenous plants which contained latex with rubber, none of these was sufficiently attractive to warrant exploitation. An introduced plant—the Madagascar rubber vine (*Cryptostegia grandiflora*)—appeared to be the one rubber-bearing plant already present in Australia in sufficient quantity to be readily multiplied and become a source of rubber, provided satisfactory methods could be developed for cultivating the plant and extracting the rubber. A survey of the naturally-occurring areas of Cryptostegia in North Queensland was therefore made by Mr. R. E. P. Dwyer, formerly Economic Botanist of New Guinea. Much of the information in this section of the report is based on Mr. Dwyer's observations.

The total acreage of the more important occurrences of Cryptostegia was computed and was found to be much less than was generally believed. Although the survey disclosed that Cryptostegia was widely distributed, the areas in which reasonably dense stands occurred were strictly limited. The largest of these areas were in the districts around Ravenswood, Charters Towers, Georgetown, and Rockhampton, where it was estimated that the total acreage would be of the order of 3,000 acres. The plant is a native of Madagascar and was introduced to North Queensland over 50 years ago. It was used as a decorative hedge plant in Charters Towers and has spread sporadically in the neighbourhood of the town. At Rockhampton it has taken posssession of a public pleasure reserve, and despite the expenditure of considerable sums by the local Council it has proved impracticable to eradicate the pest. The plant has become established naturally in the Charters Towers, Ravenswood, Georgetown, and Rockhampton districts, and it may therefore be assumed that the soil and climatic conditions in these districts are generally favourable for its growth. In all these areas there is an abundance of naturally-occurring material available for propagation. The vine appears to have a wide tolerance for soil and environmental conditions where other crops would not flourish. It may therefore be regarded as a hardy, vigorous, and drought-resistant plant. Its habit of growth appears to be influenced markedly by climatic and soil conditions. In the Charters Towers district, Cryptostegia has a shrubby habit of growth, but in the coastal areas it assumes the nature of a true vine, and the individual plants either twine around supporting trees and shrubs or form a densely intermatted growth. Once established, it spreads rather rapidly and is very difficult to eradicate owing to its extensive root development and its capacity

for throwing up fresh shoots. A feature of the plant is its ability to furnish new growth by ratoons after being cut back. There is evidence that cutting back can be repeated several times without seriously affecting the recuperative powers of the plant, but no information is available as to the ultimate limit to this procedure.

Cryptostegia appears to be very little troubled by pests and diseases, and domestic stock of all kinds avoid it. The plant responds very quickly to rain. It may be fully defoliated and yet after rain fresh shoots appear within a few days and in a month the plant is covered with foliage.

Quantitative examinations of naturally occurring Cryptostegia plants from ten different localities have been made, and the proportions of leaves, twigs, and primary and secondary stems were determined both on a freshly harvested and on a dry basis. These showed that the average percentage of leaves and young twigs (which contain most of the rubber bearing material) was 24.5 per cent and the stems 75.5 per cent. of the total weight of freshly harvested material. The average ratio of leaves and young twigs to the primary and secondary stems in naturally-occurring Cryptostegia is therefore approximately 1 to 3.

Although a perennial plant, Cryptostegia displays in the Australian environment a very definite periodicity in regard to growth, foliage production, rubber content, &c., and the leaves tend to drop off at the end of winter. Very dry and cold conditions accelerate this development and result in defoliation of the plant in the Charters Towers district. This implies that the period of the year during which the plant could be utilized as a potential source of rubber would be definitely limited. The stem is very fibrous, especially towards the base, and the twining tendencies of the plant would render its harvesting by mechanical methods difficult.

A large number of analyses of wild Cryptostegia have been made during the past year. These disclose that the leaves are much higher in rubber content than the stems, and that the rubber content both of leaf and stem varies considerably during the season. It may be said that the rubber content of the leaves at their optimum stage of development ranges from about 3 to 5 per cent. on a dry basis, or approximately 0.75 to 1.25 per cent. on the freshly harvested material. The rubber content of the stems varies from 0.5 to 0.75 per cent. on a dry basis (equivalent to about 0.12 to 0.18 per cent. on fresh, green weight).

On the basis of the foregoing figures (i.e., a leaf : stem ratio of 1 to 3, and an average rubber content of green leaf of 1 per cent. and of stem 0.15 per cent.), the rubber content of freshly harvested Cryptostegia would be 8.12 lb. of rubber per ton of green material, of which 5.6 lb. rubber would be contained in the leaf and 2.52 lb. in the stems.

The outstanding facts in regard to all naturally-occurring Cryptostegia are that, owing to its scattered and uneven distribution, and the variable age and condition of the vines, harvesting and transport costs are likely to be very high in relation to the probable rubber recovery from the vine. These costs, together with the additional costs required for the mechanical extraction of rubber, could not be met by material which contained an average of only 8 lb. of rubber per ton of harvested material. The exploitation of these naturally-occurring areas is not, therefore, economically feasible.

There is a possibility that under cultivation the rubber content of Cryptostegia may be increased, or alternatively that under cultivation the proportion of leaf—which is relatively high in rubber content in comparison with the stem—might be increased. Furthermore, the yield of Cryptostegia would no doubt be increased substantially by cultivation. These possibilities can be determined by observations made at the end of the present growing season on the Cryptostegia demonstration area which was established by the Queensland Department of Agriculture and Stock at Charters Towers in February, 1943.

It would seem unlikely that the rubber content of cultivated Cryptostegia would be substantially higher than that growing naturally in the same environment, unless a superior rubber-bearing selection had been isolated and was available for cultivation. No selection work has hitherto been carried out on Cryptostegia in Australia, and the material likely to be available for large-scale planting would necessarily be of the same genetic type as that of the acclimatized material.

There is little doubt that the proportion of leaf to stem could be increased substantially by cultivation, but there is a natural limit beyond which no such improvement would be possible. It is unlikely, however, that the average percentage of leaf could be raised much beyond 40 per cent. of the harvestable material (as compared with 25 per cent. in the naturally-occurring material). Even on this assumed high ratio of leaf, however, the total rubber content per ton of freshly harvested material would be approximately 11 lb., of which 9 lb. would be in the leaves and 2 lb. in the stems. While this would represent an improvement in the rubber content over that of naturally-occurring Cryptostegia, the yield of rubber per ton would be insufficient, at normal prices, to meet the costs of growing, cultivating, harvesting, transporting, and processing the crop.

An investigation of the problem of extracting rubber from Cryptostegia has been carried out by Messrs. F. C. Harry and W. L. Lober at the Department of Organic Chemistry, Sydney University, under the general supervision of Mr. W. E. Purnell, Rubber Technologist of the Department of Supply and Shipping. This work has made a very substantial contribution to chemical engineering knowledge of the problems of rubber extraction from plant sources. A progress report on these investigations discloses that a high percentage extraction (over 90 per cent.) of rubber from Cryptostegia leaves can be obtained by suitable chemical treatment and milling. The extraction costs, based on a yield of 100 to 115 lb. of rubber per ton of dry usable Cryptostegia (leaves and young twigs), are estimated at approximately £50 to £60 per ton of rubber. This estimate is based on the assumption that the minimum quantity of rubber to be produced that would justify the expenditure both from the chemical engineering and from a national aspect, would be of the order of 3,000 tons per annum.

In view of the fact that the rubber content of the stems is normally one-fifth to one-sixth of that of the leaves and young twigs of Cryptostegia, it would be impracticable to extract rubber from the stems. It is, however, necessary to harvest, transport, chaff, and separate the whole of the harvested green material in order to obtain the usable leaves and twigs.

The amount of rubber in freshly harvested Cryptostegia varies considerably during the season—but for the purpose of assessing the possibilities it would not, in the light of present knowledge, be safe to assume an average extractable rubber content of leaf and twig higher than 1 per cent. on a green basis (4 per cent. on oven dry material), nor a higher average ratio of leaf to stem than 1 to 2. On this basis, approximately 300 tons of green material (leaf and stem) would be required to produce 1 ton of rubber. Of this total of 300 tons of freshly harvested material, 100 tons would represent usable leaf and young twigs, and 200 tons would consist of low grade unusable stem.

No precise information is as yet available regarding the annual cost of cultivating, harvesting, and transporting Cryptostegia. Estimates have been furnished to the Council of the probable agricultural costs of Cryptostegia grown under Queensland conditions. These estimates were based on a ten-year period of productivity and on an average annual yield of 10 tons of green stems and leaves per acre from one planting. The average annual cost was given as £5 13s. per acre per annum, but this estimate was based upon the removal of the stems and leaves by mechanical means at a cost of approximately 10d. per ton for the freshly harvested material, and a transport charge of 3s. per ton from the farm to the factory. There is, however, no evidence that mature Cryptostegia can be harvested by mechanical means, and it would seem likely that the fibrous character of the lower stems and the interlocking habit of growth of the canes would preclude the application of mechanical harvesting by such machinery as is at present available. Furthermore, the estimate of transport costs of 3s. per ton appears to be exceedingly low in comparison with prevailing costs of loading and transporting other commodities, such as sugar-cane, flax, and hay.

Even on the basis of the above estimate, which assumes the possibility of mechanical harvesting and transport costs which are far below those prevailing for similar agricultural commodities, the cost of the raw material would be approximately £170 per ton of rubber produced. If to this agricultural cost is added the cost of extraction (£50 to £60 per ton of rubber), the minimum cost for raw material and extraction amounts to £220 to £230 per ton of rubber. For the reasons given above it seems certain that the cost of production would be considerably in excess of this amount.

While such a cost may not be considered excessive during a period of national emergency, there would be little possibility of an industry surviving under Australian economic conditions in competition with natural rubber from sources such as Hevea produced with cheap labour, or with guayule.

It was stated above that the minimum quantity of rubber to be produced that would justify the expenditure both from a chemical engineering and from a national aspect would be of the order of 3,000 tons per annum. Since 300 tons of freshly harvested material is required to produce 1 ton of rubber, it follows that the economic factory unit would require a minimum annual supply of 900,000 tons of raw material. To produce this amount of Cryptostegia yielding, say, 8 to 10 tons per acre per annum, it would be necessary to clear, fence, plough,

prepare, and plant, and annually cultivate and harvest, from 90,000 to 125,000 acres of land, and to transport from farm to factory nearly a million tons per annum of raw material.

The provision of the man power, implements, machinery for the clearing, preparation, fencing, fertilization, cultivation, and harvesting of, say, 100,000 acres of a crop entirely new to Australia, and the transport of one million tons per annum of harvested material for processing would make a heavy drain on Australian man power and material resources at the present juncture, with no possibility, so far as can be seen, that the industry under the prevailing costs for labour in Australia would survive in competition either with natural rubber produced with cheap labour in tropical countries, or with synthetic rubber produced in American factories.

The outstanding fact in the economics of Cryptostegia is that the freshly harvested mature material is too low in rubber content to enable rubber to be produced at a reasonable cost under Australian conditions by any process of extraction based on chemical or mechanical methods.

2. The Russian Dandelion (*Taraxacum kok-saghyz*).

The programme of investigational work on *Taraxacum kok-saghyz* (the Russian dandelion) was delayed owing to the fact that the bulk parcel of seed which was obtained from the U.S.S.R. took over seven months in transit from Kuibyshev to Sydney, and did not arrive in time for the 1942-3 sowing season. A small parcel of seed forwarded by air mail was distributed among the State Departments of Agriculture and the Division of Plant Industry, Canberra, for experimental sowings in September, 1942, and investigational work was begun in Canberra in that month. Plots were established at Black Mountain, Dickson Experiment Station, and on river soil at Fishwyck, to determine methods of sowing and spacing. The only plot which gave satisfactory germination was that sown at Fishwyck, but even here uniformity of germination was not obtained.

Trials were made to obtain a simple method of seed treatment to improve the poor germination in the field. The most important aspect of this work is to obtain a satisfactory germination in a short time, as the germination of untreated seed may eventually prove satisfactory if enough time, perhaps up to six weeks, is allowed.

There seem to be two main causes of poor germination in the field:—

(*a*) The seed as normally harvested in Russia is in various stages of maturity, and therefore under field conditions germination is slow and erratic.

(*b*) The moisture content of the soil, except when it is raining and for a brief period afterwards, is too low to obtain a satisfactory germinaton.

The sowing of the seed with superphosphate may also be a cause of poor results in the field. Experiments indicated clearly that mixing the seed with superphosphate or sowing seed and superphosphate together, which would be a normal Australian practice, has a distinctly harmful effect on germination.

The first successful seed treatment consisted simply in soaking the seed in water for 72 hours, and air-drying as rapidly as possible. Germination tests conducted a week later showed a marked improvement over untreated seed, especially when the test was conducted at low temperatures (15°C.). The chief fault with this method was that the germination of treated seed was markedly reduced on storage even for as short a period as three weeks. An experimental plot of 3½ acres was sown at the Dickson Experiment Station on the 17th of April, 1943. Seed treated by soaking in water as outlined above was used. Conditions at the time of sowing were good, but as the surface soil dried out and no rain fell for three weeks after sowing the plot failed to establish satisfactorily. As a result, further tests were made on methods of seed treatment, and the conditions required for germination.

Levitt and Ham* reported the effect of soaking the seed in solutions of various substances. The simplest method giving satisfactory results was to soak the seed for four days at 17°C. in a solution (.5M KNO_3) of potassium nitrate. This method was therefore tested with Kok-saghyz seed and gave very satisfactory results even six weeks after treatment.

An experiment was then designed to test the germination of seed treated in this way under different moisture conditions. Satisfactory germination was obtained only when the moisture content of the soil was maintained at twice field capacity. Such conditions would be found in the field only during rain and for a very brief period after the rain had ceased to fall. A second test indicated that satisfactory germination can be obtained only provided the moisture content of the soil is brought to twice field capacity and is not allowed to fall below field capacity. The soil used in these tests was Dickson loam with a field capacity of 15 per cent.

Further investigations on the relation between the moisture content of the soil and germination are being conducted, but from the information obtained it is unlikely that germination will be satisfactory if the moisture content of the soil falls below field capacity for any lengthy period. One may conclude that a high soil moisture content is essential for satisfactory germination in the field. This high moisture content must also be retained in the top layer of the soil, as the seed must not be sown to a depth greater than ½-in. to ¾-in.

In response to requests made by the State Departments of Agriculture, supplies of Kok-saghyz seed were made available for field plantings, and information from Russian and other sources regarding the technical requirements of Kok-saghyz was supplied. Field trials have been conducted under a wide variety of soil conditions in the various States. These are still in progress. Considerable difficulties have been experienced in establishing satisfactory stands in most localities, and the general results of the preliminary experiments are disappointing. The most comprehensive series of preliminary tests was reported from South Australia, where the Department established sowings in 1942 at 14 localities in the Mount Lofty Ranges and in the south-east of the State. There was a wide variation in the average weight and rubber

* *Plant Physiol.* **18** (2): 288-293, 1943.

content of the roots of Kok-saghyz grown in the different experimental areas. The average rubber content of the roots at the 14 centres was approximately 6 per cent. on a dry basis with plot averages of over 9 per cent. at several centres.

The Department of Agriculture of South Australia, in reporting these results, considered that the indications at six of the centres were sufficiently promising for the Department to arrange with private farmers for the sowing of demonstration plots. The Council undertook to share with the Department of Agriculture the cost of establishing, fencing, and harvesting these demonstration areas.

The preliminary investigational work with Kok-saghyz has shown:—

(1) Some form of pretreatment of the seed is essential prior to sowing. This pretreatment may take the form of prolonged soaking in water; partial pre-sprouting and drying of the seed; a form of "vernalisation" (i.e., exposure of moistened seed to prolonged low atmospheric temperatures) such as is practised in Russia; or soaking the seed with dilute mineral nutrients.

(2) A high moisture content of the surface layers of soil is essential during late spring or early summer when the seed is sown. Good spring rains are therefore necessary for successful establishment.

(3) A high degree of soil fertility is essential for the satisfactory growth and development of the plant. The most satisfactory production is obtained from soils rich in organic matter.

(4) The roots of the plant are relatively small and, though the rubber content appears to be satisfactory, only moderate quantities of rubber per acre are likely to be obtained.

3. Guayule (*Parthenium argentatum*).

Prior to the outbreak of war in the Pacific, investigations on guayule in Australia had been limited to observations on its growth since 1932 in the Plant Introduction Garden at Canberra. The plants at Canberra set viable seed each year, but the total stock of seed available in 1942 was limited to a few pounds. In July, 1942, advice was received from the United States Department of Agriculture that Congress had authorized the planting of an area of 75,000 acres of guayule. The areas considered to be most suitable for the propagation of the plant were in south-western U.S.A., and mainly in Southern California, where climatic conditions, particularly in respect to rainfall distribution, were somewhat similar to those in certain parts of South Australia.

In view of the gravity of the rubber position as it appeared in 1942 and the programme then envisaged in the U.S.A., efforts were made to secure a stock of seed for investigational work and also for the establishment of a commercial demonstration area on the basis of the knowledge received from overseas. Through the courtesy of the U.S. Rubber Emergency Project, a bulk parcel of freshly harvested seed from the Salinas (California) plantations was made available and arrived in Australia in October, 1942. Investigations on nursery technique and

field establishment were carried out by the Division of Plant Industry at Canberra (A.C.T.); by the Waite Institute (University of Adelaide) in South Australia; and also by the Department of Agriculture of Western Australia.

The major portion of the work was carried out in South Australia by Professor H. C. Trumble and Mr. R. L. Crocker, and much of the information in this section is based on progress reports furnished in respect of the South Australian project. The production of seedlings on a commercial scale has been shown to require very special conditions of seed and nursery treatment, some of which were not sufficiently evident from the advice received from overseas to ensure efficient nursery production at the outset. Moreover, it was not apparent till the autumn and early winter of 1943 that the plant is very sensitive to low temperatures. Growth appears to cease completely below 60°F. and is not active between 60° and 70°F.

Serious difficulties were experienced through the low viability of the seed received from the U.S.A. This necessitated the carrying out of an intensive short-term programme of laboratory and field investigations of the factors affecting the germination and early growth of guayule, and it was not until February, 1943, that a satisfactory technique was developed to overcome these difficulties. Notwithstanding these problems, over one million hardened seedling plants were raised in the nursery in Adelaide. Sufficient material was available for field plantings amounting to 102 acres in South Australia. The bulk of the plantings was made on irrigation land, but numerous experimental plantings were made on dry land areas throughout the State. In addition, 17 acres were planted at the Dickson Experiment Station, Canberra, from material raised by the Division of Plant Industry in nurseries at Yarralumla and in the Divisional glasshouses at Canberra. Small experimental sowings were made in Western Australia, New South Wales, and Queensland.

The results of the investigations on seed treatment disclosed that a high percentage of nursery establishment could be obtained by a combination of four treatments, namely (*a*) prolonged mechanical agitation of the seed in running water, (*b*) further mechanical agitation of the seed in a 5 per cent. chloride of lime soluton, (*c*) centrifuging to remove excess water, and (*d*) pre-sprouting at 20°C. On a nursery area of slightly less than half an acre, planted in October, 1943, over half a million seedlings have been raised by using this treatment.

The establishment of guayule in the field during 1943 was generally disappointing, particularly on the dry land areas. A large number of factors were responsible for this result, including initial inexperience with transplanting machines, unsatisfactory soil type, failure of the plant to make any winter growth, weed infestation, time of planting, and age of seedling. It was found that age of seedling and time of planting were very important factors in establishment and in coping with the competition of weeds, and these factors are being thoroughly investigated.

The greatest difficulties in field plantings in South Australia arose from the exceptionally long cold winter, the extreme weediness of South Australian cereal lands generally, and the acute man-power shortage

throughout rural areas. The progress of the work has demonstrated that thorough long-term preparation of the land, including deep working and fallowing and total elimination of weed growth, is desirable, and that the optimum time of planting the hardened seedlings is towards the end of winter or early spring. The climatic conditions of parts of southern Australia appear to be suitable for the growth of guayule, and extensive areas of soil on which the plant may be grown occur in the agricultural areas.

It is as yet too early to assess the commercial possibilities of the crop under Australian conditions. Yield per acre and rubber content comparable with those observed in California at corresponding stages of growth have been obtained in the experimental areas. However, while the climatic and soil conditions appear to be satisfactory for the growth of guayule, the major difficulties associated with its development are likely to be those relating to man-power problems and the cost of raising the final product.

Whether the crop can be grown on a large scale as a post-war commercial undertaking will depend upon the efficiency with which the mechanization of the various necessary operations can be achieved and on the result of further investigations into the means of improving both the yield and the rubber content of the crop. It is evident from the experience already gained that the plant can be grown successfully either on irrigated or on non-irrigated land, but the present necessity for nursery production of seedlings, their transplantation to the field, thorough initial cultivation, later inter-row cultivation to control weeds, and the fact that probably three to five years may be required to yield the product, indicate that the final costs may be relatively high compared with those of other agricultural crops.

Under irrigated conditions the crop must be regarded more as a horticultural crop than an agricultural one, and if its value per acre is substantially less than that of standard horticultural crops it suffers a disadvantage. The development of the plant under irrigation has been pursued mainly because of the fact that in the U.S.A. guayule under irrigation can be harvested in two years after establishment. There is a possibility, based on the results of experimental trials in South Australia, that a method of seeding might be devised which would enable the nursery side of the project to be dispensed with. This, if proved feasible, would represent a substantial advance in reduced costs of establishment, and in production costs.

The scientific investigation of guayule should be pursued with vigour in order to assess its productive capacity under Australian conditions and to determine with accuracy the numerous technical, practical and economic factors involved in its cultivation.

4. Synthetic Rubber Research.

Dr. I. W. Wark, Chief of the Division of Industrial Chemistry, has reported on the progress of investigations on the production and use of thiokol, and the production of 2:3-butylene glycol from grain and its conversion into butadiene.

" On the fermentation side Mr. W. G. Crewther is now investigating methods of production of 2 : 3-butylene glycol from grain, using species of organisms specially obtained from North America. This work is being carried out in the Section of Biochemistry under the supervision of Dr. F. G. Lennox.

" Arrangements have been made for a study of the methods of production of butadiene from 2 : 3-butylene glycol in the Organic Chemistry Department of the Melbourne University. This project is a joint effort between that Department and the Organic Section of this Division. The work is being supervised by Associate-Professor W. Davies, and the Division has provided the services of Dr. M. E. Smith and will also provide a laboratory assistant. As an initial step, Dr. Smith is investigating the recovery of 2 : 3-butylene glycol from the fermentation liquors. Considerable progress has been made in assembling and testing the necessary apparatus. It can be said that this whole investigation is well under way."

A Report on Live-Bait Fishing for Tuna in Australia.

*By A. Flett.**

The Californian live-bait fishery for tuna is one of the most interesting and important practical fishery developments in America, and perhaps, from the popular point of view, the most spectacular kind of fishery carried on anywhere. Though of fairly recent origin, this method of fishing for tuna provides the great bulk of that fish which is processed by Californian canneries. A full description of the fishery, with an account of the boats and gear employed, is given by Godsil (1938)†, and a brief summary has been provided by Serventy (1941)‡.

Gear Used on M.V. "Warreen."

During the early part of *Warreen's* investigations on live-bait methods for tuna fishing in south-eastern Australia, a wooden bait tank, 5½ ft. by 4½ ft. by 5 ft., was carried on the afterdeck, port side. When filled to capacity, it weighed approximately 4 tons, and gave a considerable list to the vessel, as well as proving a hindrance to normal activities. After the removal of the pilchard purse-seine, the tank was carried on the turntable; working from the turntable was found to be extremely awkward, and during the overhaul of April, 1940, the table was removed, leaving a clear afterdeck for the bait tank and the lampara net used for catching the bait.

The tank was built of 1½ in. by 8 in. Oregon, caulked in the seams, and bolted together. At first it was made in removable sections to facilitate stowage in the hold, but when this proved impracticable, it was strengthened by hardwood girders and bolts, being made into a permanent structure. The water entered the tank through two holes on top, and was diverted to two of the sides by boards inside the tank top. After several experiments, these boards were finally filled with ½-in. holes to break the force of the water. A screen of wooden laths, ⅛ in. apart, was placed on the side opposite to where the water entered, to prevent fish reaching the discharge pipe. This pipe was controlled by a valve, enabling the height of water in the tank to be regulated. There was an opening on the tank top, approximately 2 feet square, fitted with 9-in. high coamings, but this height proved insufficient to prevent the water "slopping" in the tank, therefore, removable coamings had to be fitted when the vessel was at sea.

Water was supplied from a 3-in. centrifugal pump, driven by the auxiliary engine, and delivered to the tank by two 1½-in. diameter rubber hoses. A small portable motor and centrifugal pump was substituted when the auxiliary engine was shut down in port or at anchor.

* Formerly Master, M.V. *Warreen*, Division of Fisheries.

† Godsil, H. C. (1938).—The high seas tuna fishery of California. Calif. Div. Fish Game, Fish Bull. No. 51.

‡ Serventy, D. L. (1941).—The Australian Tunas. Coun. Sci. Ind. Res. (Aust.), Pamph. No. 104.

In use, these arrangements proved satisfactory, and on two occasions bait was carried in the tank for nine and ten days respectively, from Jervis Bay to Hobart, and was liberated at the latter port in splendid condition. Among the major causes of death was the entry of free air with the water. This is fatal to bait fishes and must be guarded against at all times; leaking packing glands on the pump are the main cause of the trouble.

Live-bait fishing gear of Japanese manufacture, as used in the Californian fishery, was imported and used in all the experiments.

Iron fishing racks, on which the fishermen stood, were suspended over the ship's side and stern, just above water level, and from these fishing was carried out. Usually six men comprised the fishing crew.

Bait was caught sometimes by means of a lampara net, and at other times by a 6-ft. diameter hoop net lowered into the water and drawn rapidly to the surface when a shoal of bait fish came near a light suspended over the net. This bait was kept alive in the tank until tuna were located, when it was thrown overboard in handfuls to attract the tuna alongside the vessel.

In most respects, therefore, *Warreen* thus fitted was a practical bait boat. Her capabilities were severely limited, however, by the capacity of the bait tank, which proved altogether insufficient and, as will be seen later in this report, was a material restrictive factor in the experiments.

The Live Bait Experiments.

(i) *December,* 1938.

During the southern bluefin (*Thunnus maccoyii*) season of 1938, when the fish were very plentiful around Montague Island, N.S.W., several attempts at live-bait fishing were made, but all proved unsuccessful.

The bait used was scad—or "yellowtail" of N.S.W.—(*Trachurus declivis*), caught alongside the wharf at Eden by hoop net. These fish when liberated immediately sought shelter under the vessel's hull. Southern bluefin were around the vessel, but none were caught by poles, though they were freely taken by trolling.

(ii) *February,* 1940.

The presence of anchovies (*Engraulis australis*) in Towfold Bay was used to test the live bait possibilities of fishing for striped tuna. During this month was made the most successful of all *Warreen's* experiments in this field.

The anchovies were 3 to 3½ inches in length, and were caught by a hoop net used in conjunction with a submarine light worked off the ship's batteries. Enough bait was caught on January 30 to fill the bait tank, some scad caught similarly being added. Bad weather delayed *Warreen* for two days, during which the bait lived well in the tank. On February 1, large shoals of striped tuna were encountered 15 miles S.W. from Gabo Island, some of them being acres in extent. The weather was fine and calm. The ship was stopped at 3 p.m. amongst the tuna, which came alongside, taking the live bait freely, although

they would not take the feathered lures. Six tuna were caught with live bait on barbless hooks from the shoals, which stayed alongside for $1\frac{1}{2}$ hours. The scad again proved useless as bait, as they dived deep immediately they were liberated. After emptying the tank *Warreen* returned to Eden.

On February 3, the lampara was shot around a flock of gannets and terns which were diving in shallow water (4 fathoms), no anchovies being seen on the surface. However, approximately one ton was caught in the net, and of this 35 dipnetfuls (roughly each dipnetful holds 8 lb. of fish) were placed in the bait tank, the balance being liberated. The striped tuna were located the following day between Cape Howe and Gabo Island. One shoal of tuna followed the vessel, but though they took the bait freely, they would not come close to the ship. Another shoal, about one mile distant, was, however, brought alongside, and in four hours 32 striped tuna and one southern bluefin were caught. These fish were taken on feathered "squids" (hooks) and hooks baited with live anchovies. Whilst fishing was at its height the shoal was scared away by a tuna breaking a bamboo pole and pulling it through the water.

As a strong southerly breeze was blowing, *Warreen* returned to Eden, and sheltered there until February 7. During this period, the anchovy bait lived well in the tank. On February 7, a large shoal of anchovies came around the submarine light, and were caught by the hoop net, the bait tank being filled with 65 dipnetfuls. This, in view of later experience, was more than the tank could carry for any length of time (the proved capacity was found to be about 45 dipnetfuls).

Striped tuna were encountered in Disaster Bay and pole-fishing was resumed. From the first school, which did not bite very well, nine fish were caught in one hour. Another school located one mile distant yielded 74 striped tuna in one hour's fishing, when the bait was exhausted. A fresh southerly breeze was blowing during this time, and the water was rough—the tuna appeared to bite well under these conditions, since the disturbed water obscured the lines and ship.

(iii) *May,* 1940.

On May 11, the bait tank was filled with Tasmanian sprats (*Clupea bassensis*) which were caught by lampara net in Great Taylor Bay, Bruni Island, Tasmania. The fish scaled very easily in the net when it was being hauled, and next day most of the sprats in the tank had died. With the dead and dying bait, however, an attempt was made at pole-fishing on the southern bluefin at Cape Pillar, Tasman Island. Only one tuna was caught, although they were fairly plentiful and numbers were trolled by another fishing boat in the vicinity. The tuna came around the ship and took the dead bait freely as it sank in the water, but bait has to be lively to attract tuna to the surface.

Variations in volume of water flowing through the bait tank and a rough passage to Cape Pillar were at the time held responsible for the heavy mortality among the sprats, but subsequent tests (May, 1941) under ideal conditions proved that these fish would not live in captivity, either in a bait tank or in a well such as is used on Tasmanian fishing vessels.

(iv) *June*, 1940.

On June 29 at Coles Bay (in Oyster Bay), Tasmania, the bait tank was filled with anchovies taken with submarine light and hoop net, but fully three-quarters of them were dead next day when a test of pole-fishing on southern bluefin was made at St. Helens, Tasmania. In one hour's fishing by four men, live and dead bait being thrown overboard, several tuna came alongside the ship, four being caught. The tuna were not very plentiful at the time but they could be seen taking the dead bait as it sank in the water.

The high mortality of the bait appears to have been due to the excessive handling and subsequent buffeting it received in the tank while the *Warreen* was steaming to St. Helen's in rough seas. The anchovies were taken in very small lots by hoop net over a period of two hours.

(v) *October*, 1940.

The cruises during this period were devoted primarily to testing out the pole-fishing method on the southern bluefin shoals which visit the south coast of New South Wales in the spring and early summer. Pilchards (*Sardinops neopilchardus*) were used as bait, no anchovies in any quantity being met with on the New South Wales coast this season.

Difficulty was experienced in procuring bait, and after fruitless attempts in Jervis Bay and elsewhere on the south coast, *Warreen* proceeded north to Port Stephens where the tank was filled on October 10 with small 2-in. pilchards taken with submarine light and hoop net in Fly Roads. The first attempt at pole-fishing was made on October 12, near Montague Island. Bluefin tuna were plentiful but they would not approach the ship. Eight different shoals, located by trolling, were tried in succession but all behaved similarly. The trolled examples were found to be full of " small feed ", approximately similar in size to the pilchard bait and including bellows-fish, small leatherjackets, etc., and various crustaceans such as squillid-larvae and krill. On the following day, October 13, seven shoals of bluefin were tried south of Montague Island, when the bait tank was exhausted without any tuna being induced to come alongside the ship. The small pilchards lived well in the tank during the period.

The tank was next filled on October 25 when large shoals of pilchards were found at the entrance to Jervis Bay during the early morning. A haul of the lampara netted two tons of 5-in. pilchards, and after filling the bait tank the balance of the catch was liberated. Bluefin and striped tuna were located near Brush Island later the same day, and in $1\frac{1}{2}$ hours' pole-fishing 60 southern bluefin were caught. These shoals were not large, however, and the fish did not bite freely. Nevertheless, the test showed conclusively that bluefin could be caught by this method.

As tuna were known to be in the near vicinity of the bait-ground, the tank was filled with bait to a greater capacity than previous experience had shown to be desirable, and a considerable portion of the pilchards died within eight hours of capture through overcrowding. A fresh north-easterly breeze was blowing during the fishing tests, and the sea was fairly rough.

(vi) *December,* 1940.

Many large shoals of pilchards were located in Jervis Bay on December 8, and the bait tank was filled by a lampara haul. No opportunity was found of using these either on southern bluefin or striped tuna, and the bait was eventually liberated in Blackman's Bay Tasmania, after living in a healthy condition for nine days. On the day of capture, however, whilst *Warreen* was tied up at the jetty at Jervis Bay, a quantity of dead pilchards was thrown overboard and a school of bonito (*Sarda australis*) was attracted alongside. In one hour 35 fish were landed with poles.

(vii) *April,* 1941.

An attempt was made to pole-fish striped tuna in Tasmania with pilchards caught by lampara net at Jervis Bay, N.S.W. The tuna were tried off St. Patrick's Head on April 30, but only three fish were caught. The tuna ate the bait, but would not take the lures or approach closely to the ship. This particular shoal of striped tuna was very small and scattered. The pilchards in the bait tank lived very well, the mortality being half a dip-net daily. The survivors were liberated in a healthy condition ten days after capture.

Discussion of Results.

These series of tests, though they provided no spectacular results, indicated that two species of tuna in local waters, the striped tuna and the southern bluefin, can be taken by pole-fishing, though on present evidence, the former gives a greater promise of commercial importance. From the bait aspect, the tests proved that anchovies and pilchards, the latter particularly, were suitable bait, both species living well in the tank, the pilchards having been carried 500 miles in a wide range of water temperatures and living up to 9-10 days, when they were liberated in a healthy condition.

The supply of bait is the crucial factor in this method of tuna fishing. In the experience of *Warreen,* bait proved hard to capture, and was very sporadic in its appearance. Of the two bait species available, pilchards proved the more reliable as far as occurrence was concerned, that species being found along the New South Wales coast in bays and estuaries during the tuna season. Anchovies were not met with in sufficient quantity other than in the 1939-40 season. In general the occurrence of anchovies along the New South Wales coast seems subject to very considerable fluctuations from year to year.

Best results with actual tuna fishing were obtained during fairly rough weather and from shoals whose presence was indicated by tuna caught on the trolling gear. Most shoals actually seen on the surface and tried with live bait did not come close to the fishing racks. The experiences of fishermen in southern Californian and Mexican waters is that many shoals of tuna may be tried before a shoal is found which will "bite" freely, and our experience in Australian waters proved similar. Godsil, in the publication already mentioned, writes (pp. 29-30): "On an average trip, a crew will count upon two or three 'good' days of fishing with a daily catch of 20 to 50 tons. The balance of the catch is less sensational and at times, heart-breakingly slow. . . . For days on end a boat may cruise through schools of tuna and hardly

get a strike. . . . Every school must be tried and the days are spent clambering in and out of the fishing racks. . . . (and only a miserable half dozen or so fish taken). . . . From dawn to dark this goes on, and a week of this will weary any one. In the log book of a tuna boat, will be seen repeatedly the statement . . . 'Lots of fish—won't bite!'"

The capacity of *Warreen's* bait tank was very inadequate, and in consequence the bait supply was quickly exhausted after a short spell of fishing, after having often taken weeks to procure. A regular Californian bait boat with large bait capacity carries sufficient to try out many shoals of tuna; *Warreen* was restricted to a few hours' fishing, and it was impossible to persevere with refractory shoals, until one met with, say, a school of the type which gave good results on February 7, 1940. Owing to limited deck space, no larger tank could be carried on the vessel.

A Method of Coping with the Bait Problems.

A plentiful supply of bait which can be procured as required is an essential to successful live-bait fishing. Under conditions as disclosed over a period of four years, when a look-out was constantly kept for bait, it was found to be impossible to fulfil this requirement. The solution appears to be to capitalize the occasions when the sporadic shoals of bait fish become available to the fishermen, and to hold them in some system of pens or tanks to be fed to the tuna fishing boats as needed. Local conditions strongly suggest that it may be necessary to divide the live-bait tuna fishery, which is carried on by single units in California, into two distinct fisheries in south-eastern Australa.

It is suggested here that, when bait is available, it could be caught by lampara or other gear and transferred to wooden containers moored in a sheltered part of a bay or estuary to which the tuna boats could go to replenish their bait tanks. This method was used in the Philippines prior to the war, and also in Mexico, where boats were supplied daily with fresh bait. The Philippines bait fishery, carried on as an adjunct to live-bait tuna fishery, is described fully in an illustrated article by Jose S. Domantay (1940)*.

Pilchards are present on the New South Wales coast in large quantities during the tuna season, but are very intermittent in their appearance at the surface. Most attempts to capture them in the day-time with a lampara net proved unsuccessful, owing to the speed with which they dart through the water and dive deep at the slightest scare, even a bird's approach being sufficient to disturb them. A small-mesh purse-seine offers the best opportunity of capture at night, but difficulty will be experienced in transporting the fish from the open sea to the "pans." If the bait is seined direct from a vessel with tanks suitable for carrying it, much of the difficulty will be overcome, since towing bait is necessarily a slow process and subject to weather conditions.

A source of live-bait supply which warrants attention is the anchovy in Port Phillip Bay. These fish seem to occur regularly and in reasonable abundance from October to May, which includes the striped tuna season.

* Domantay, J. S. (1940).—The catching of live bait for tuna fishing in Mindanoa. *Philipp, J. Sci.* 73 (3): 337-343.

NOTES.

Council of Scientific and Industrial Research, India.

The organization of industrial research in India, with a view to making her industrially self-sufficient, has been engaging the attention of the Government of India for some time. An Industrial Research Bureau had been established as a clearing house of industrial intelligence after the Sixth Industries Conference in 1934, but at the outbreak of war many sources of supply of finished products to India were either stopped entirely or much curtailed, and it became apparent that a central scientific and industrial research organization should be established immediately. Thus the Board of Scientific and Industrial Research was set up in April 1940, for a period of two years in the first instance.

The functions of the Board were to advise the Government as to the lines on which scientific and industrial research should be conducted, particularly as regards industries whose importance and possibilities were brought into the foreground by war-time conditions. The Board was to utilize and co-ordinate the work of existing organizations and to make recommendations to the Government concerning the general lines on which industrial research should be pursued, and the specific problems which might be assigned to the technical staff directly under the control of the Board on the one hand and to the various University and other scientific institutions on the other. The Board consisted of four scientists, seven industrialists, and one departmental head, with the Executive Council Member in charge of the Commerce Department as chairman. It was to meet every three or four months. Research committees, composed of scientists and industrialists, were set up to examine and report on research schemes and watch their progress. Nineteen such committees were set up in the first two years.

When certain researches had reached a stage at which their commercial exploitation could be considered, an Industrial Research Utilization Committee was formed to settle the terms on which the researches could be released to manufacturers. The Committee, consisting mostly of industrialists, was under the Board and contained representatives of industries and the Chamber of Commerce.

In 1942, the Council of Scientific and Industrial Research was constituted to co-ordinate and generally exercise administrative control over the Board of Scientific and Industrial Research and the Industrial Research Utilization Committee. Its 14 members are drawn mostly from the Board and the Committee. The Council has been established on a permanent basis and is financed by the Industrial Research Fund, providing in the first instance an annual grant of 10 lakhs of rupees (about £75,000 sterling) for five years.

Since its formation, the Board has been mainly occupied with the solution of urgent war problems, but it has devoted considerable attention to the organization of scientific and industrial research on a scale commensurate with India's expanding needs. Plans have already been prepared for establishing a National Chemical Laboratory, a National Physical Laboratory, a Metallurgical Research Institute, a Central Glass and Silicates Research Institute, and a Central Fuel

Research Station. When these are given effect to, India will be provided with facilities for research reasonably adequate to meet her immediate requirements.

In order to give publicity to the research activities initiated by or undertaken at the instance of the Board, it was decided to publish the *Journal of Scientific and Industrial Research*, the first number of which appeared in October, 1942. The Journal is intended primarily for the publication of researches in applied sciences. Its scope is broad and includes technical articles, reviews of recent developments in industrial research and of scientific and technical books, abstracts of research papers, and notes on industrial products and on patent literature. Results of laboratory investigations having a direct bearing on the development of industries are published in the "Letters to the Editor" section, and enquiries on industrial processes and developments are answered through the medium of the Journal. Publication is quarterly, and copies may be obtained on application to The Secretary, Council of Scientific and Industrial Research, New Delhi, India. The annual subscription is 6 rupees.

Recent Publications of the Council.

Since the last issue of the *Journal* the following publications of the Council have been issued:—

Bulletin No. 168.—"A Survey, Census, and Statistical Study of the Horticultural Plantings on the Murrumbidgee Irrigation Areas, New South Wales," by A. Howard, M.Sc., A.A.C.I., and G. A. McIntyre. B.Sc.

After the wet winter of 1939 had caused extensive flooding in the areas and killed many valuable citrus and other trees, the Irrigation Research Extension Committee decided to make a survey to determine the extent of the damage, and gather other useful information. Accurate statistics were wanted of the damaged and undamaged trees, including their variety, age, and history. Also, the need for a survey of the whole horticultural plantings had long been felt, since it was necessary to have detailed information on layout of plantings, irrigation structures, and cultural methods used, to serve as a basis for research and advisory work.

The results were analysed to determine if the environmental conditions and cultural treatments had any effect on the health of the different varieties of trees, and to show the ability of the different varieties to withstand the 1939 winter. The effect of most factors, even of such important ones as the use of nitrogenous or organic manure or the presence of a high watertable, was not found to be outstandingly great, but there was general agreement between the crops. The large variability between farms similarly classified on all the factors under consideration in each analysis brings home the impossibility of arguing from a few isolated instances. Statements of the value or otherwise of a certain treatment are often made on the basis of a single comparison between plantings on widely separated farms. It may safely be said that if differences due to different treatments have not been shown in the present survey, they are either non-existent or can only be demonstrated by carefully controlled experiments.

Bulletin No. 169.—"The Entomological Control of St. John's Wort (*Hypericum perforatum* L.) with Particular Reference to the Insect Enemies of the Weed in Southern France," by Frank Wilson.

The apparent failure to establish certain British *Hypericum* insects in Australia led to an investigation of the insect enemies of this weed in Southern France, where the climate is similar to that in south-eastern Australia.

The Bulletin deals in some detail with the distribution of the *Hypericum* insects, the life-histories of those occurring in the Var, their parasites and other limiting factors, their effect upon the host plant, and other matters of a similar nature. Various problems connected with the theoretical basis for attempts to control alien weeds by means of phytophagous insects are discussed, largely on the basis of evidence derived from the study of the St. John's wort insects, and the conclusions are summarized.

Three insect enemies of St. John's wort have now been established in Australia. *Chrysolina hyperici* was imported from England and, after liberation, disappeared for some years, but reappeared in 1939 in large numbers at Bright, Victoria, where it has become increasingly common. It occurs in densely populated colonies which rapidly defoliate the host. *Agrilus hyperici* and *Chrysolina gemellata* were imported from France and liberated in 1939. They have both become established but have not yet had time to exert a very great effect upon the wort. It is hoped that these three insects will later give a useful degree of control of the weed.

Bulletin No. 170.—"Pea Mosaic on *Lupinus varius* L. and other Species in Western Australia," by D. Norris, B.Sc.(Agric.).

This Bulletin describes the symptoms produced on five species of lupin by the virus of pea mosaic. On *L. varius* the symptoms are unusual, the plants entering a secondary phase of growth after infection. Although seed transmission of the virus is suspected to occur in peas, it does not occur in lupins or subterranean clover. The disease is transmitted to lupins mainly from peas, broad beans, and sweet peas, but the shrub *Cassia corymbosa* is recorded for the first time as a perennial host of the virus, enabling it to survive the dry summer. Nine species of aphid are shown to be able to transmit the virus. In Western Australia the common peach aphid, *Myzus persicae,* is the most important field vector. The disease appears to be of little economic significance.

Bulletin No. 171.—"Experiments with Insecticides against the Red-legged Earth Mite (*Halotydeus destructor* (Tucker))," by K. R. Norris, M.Sc.

This Bulletin describes preliminary tests in a programme aimed at the control of the red-legged earth mite. The work was discontinued because of the war.

Laboratory and field experiments were carried out at Katanning, Western Australia, where the mite is a serious pest of the pastoral areas. A new method of controlling the mite by means of poison baits was developed, depending for its effectiveness on the tendency of the mite to suck up sugar solution. A solution of cane sugar and a poison

was used to moisten cereal chaff, which was then scattered over the infested area. Sodium arsenate was shown by field and laboratory experiments to be the most satisfactory poison. This method of control, though uneconomical for use on pastures, should find a definite application in the control of mites in vegetable gardens.

The most satisfactory sprays tested were a preparation containing lauryl thiocyanate, a rotenone-white oil emulsion, and nicotine sulphate sprays. The most effective dusts were preparations of nicotine. The relatively good results obtained with dusts prepared from waste Western Australian tobacco leaf mixed with slaked lime show that the material could be employed for mite control. D.N. dust (containing dinitro-o-cyclohexylphenol) also proved of some value.

Industrial Chemistry Circular No. 4.—" Separation of Ergot from Rye Corn," by Enid C. Plante, B.Sc., and K. L. Sutherland, M.Sc.

This Circular describes in detail a flotation method for separating ergot from rye corn and gives plans of the necessary separation tank and pneumatic flotation machine. The surface of rye grain is wax-like, and in water air bubbles cling to it, but not to ergot. By treating the grain with paraffin emulsion, the oiliness of the surface is increased, and when the grain is subsequently immersed in water and a stream of air passed through it, the bubbles carry the grain up while the ergot sinks; the grain can then be skimmed off. The process includes immersion in a salt solution of specific gravity 1·12 to 1·13 before and after the flotation, and as a result ergot 99 per cent. pure is obtained.

Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

Bulletin No. 172.—" Zebu-Cross Cattle in Northern Australia. An Ecological Experiment," by R. B. Kelley, D.V.Sc.

Bulletin No. 174.—" Recent Advances in the Prevention and Treatment of Blowfly Strike in Sheep," Supplement to Report No. 2 by the Joint Blowfly Committee.

Bulletin No. 175.—" The Recovery of Inter-block Information in Quasi-Factorial Designs with Incomplete Data. 2. Lattice Squares," by E. A. Cornish, M.Sc., B.Agr.Sc.

Bulletin No. 176.—" The Analysis of Cubic Lattice Designs in Varietal Trials," by I. F. Phipps, M.Sc., B.Agr.Sc., Ph.D., A. T. Pugsley, B.Agr.Sc., S. R. Hockley, and E. A. Cornish, M.Sc., B.Agr.Sc.

Bulletin No. 177.—" A Soil Map of Australia," by J. A. Prescott, D.Sc., A.A.C.I.

Bulletin No. .—" Lubrication between the Piston Rings and Cylinder Wall of a Running Engine," by J. S. Courtney-Pratt, B.E., and G. K. Tudor, B.E.

PLATE 1.

Estimation of Damage to Potato Foliage by Potato Moth.

(See page 30.)

Standards for moth damage rating, showing the condition of plants rated 3, 4, and 5. Damage to plants rated 2, 1, and 0 is progressively less severe.

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